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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/14420 A2

(51) International Patent Classification[7]: C07K 14/00

(21) International Application Number: PCT/US00/23365

(22) International Filing Date: 25 August 2000 (25.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/150,576 25 August 1999 (25.08.1999) US

(71) Applicants *(for all designated States except US)*: **UNIVERSITY OF TORINO** [IT/IT]; Department of Biomedical Sciences and Human Oncology, IRCC, SP 142, I-10060 Candiolo (IT). **REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 12th floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ARTIGIANI, Stefania** [IT/IT]; Corso Brunelleschi, 121/B, I-10100 Torino (IT). **COMOGLIO, Paolo, M.** [IT/IT]; Strada Valsalice, 183/8, I-10100 Torino (IT). **GOODMAN, Corey, S.** [US/US]; Regents of the University of California, 12th floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US). **TESIER-LAVIGNE, Marc** [US/US]; Regents of the University of California, 12th floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US). **TAMAGNONE, Luca** [IT/IT]; Corso Einaudi, 43, I-10129 Torino (IT).

(74) Agent: **COX, Niki, D.**; Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *Without international search report and to be republished upon receipt of that report.*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/14420 A2

(54) Title: NOVEL PLEXINS AND USES THEREOF

(57) Abstract: The invention provides methods and compositions related to novel plexins. The polypeptides may be produced recombinantly from transformed host cells and from the disclosed plexin encoding nucleic acids or purified from human cells. The invention provides isolated plexin hybridization probes and primers capable of specifically hybridizing with the disclosed plexin genes, plexin-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in biopharmaceutical industry. The invention also provides novel plexin neuropilin multimeric receptor complexes for semaphorins and methods of use thereof, including but not limited to, the treatment and diagnosis of neurological disease and neuroregeneration, immune modulation, and viral and oncological diseases.

# NOVEL PLEXINS AND USES THEREOF

## BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to the identification and characterization of four novel proteins that are members of the plexin family.

Summary of the Related Art

Plexin family members encode large transmembrane proteins, whose cysteine-rich extracellular domains share regions of homology with the Scatter Factor receptors (encoded by the Met gene family). The extracellular domains of plexins also contain ~500 amino acid Semaphorin domains (see below). The highly conserved cytoplasmic moieties of plexins (approx. 600 amino acids), however, have no homology with the Met tyrosine kinase domain, nor with any other known protein. Met-like receptors and their ligands, the Scatter Factors, mediate a complex biological program including dissociation of cell-cell contacts, motility and invasion (for a review see Tamagnone, L. and Comoglio, P.M. (1997) "Control of invasive growth by hepatocyte growth factor (HGF) and related scatter factors." Cytokine Growth Factor Rev *8*, 129-142). During embryogenesis Scatter Factor-1 and Met promote the dissociation of cell layers in the somites and drive the migration of myogenic cells to their appropriate location (Bladt, F., Riethmacher, D., Isenmann, S., Aguzzi, A., and Birchmeier, C. (1995) "Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud." Nature *376*, 768-771; Maina, F., Casagranda, F., Audero, E., Simeone, A., Comoglio, P.M., Klein, R.a., and Ponzetto, C. (1996) "Uncoupling of grb2 from the met receptor in vivo reveals complex roles in muscle development." Cell *87*, 531-542). Met and Scatter Factor-1 are also involved in controlling neurite outgrowth and axonal guidance (Ebens, A., Brose, K., Leonardo, E.D., Hanson, M.G.J., Bladt, F., Birchmeier, C., Barres, B.A., and Tessier-Lavigne, M. (1996) "Hepatocyte growth factor/scatter factor is an axonal chemoattractant and a neurotrophic factor for spinal motor neurons." Neuron *17*, 1157-1172; Maina, F., Hilton, M.C., Ponzetto, C., Davies, A.M., and Klein, R. (1997) "Met receptor signaling is required for sensory nerve development and HGF promotes axonal growth and survival of sensory neurons." Genes Dev *11*, 3341-3350; Maina, F., Hilton, M.C., Andres, R., Wyatt, S., Klein, R., and Davies, A.M.

(1998) "Multiple roles for hepatocyte growth factor in sympathetic neuron development." Neuron *20*, 835-846).

The first clue regarding a possible function for plexins came from the finding that a novel plexin, Vespr, interacts with the viral semaphorin A39R (Comeau, M.R., Johnson, R., DuBose, R.F., Petersen, M., Gearing, P., VandenBos, T., Park, L., Farrah, T., Buller, R.M., Cohen, J.I., Strockbine, L.D., Rauch, C., and Spriggs, M.K. (1998) "A poxvirus-encoded semaphorin induces cytokine production from monocytes and binds to a novel cellular semaphorin receptor, VESPR." Immunity. *8*, 473-482). Semaphorins are a large family of secreted and membrane-bound molecules that are characterized by an extracellular domain containing a ~500 amino acid Semaphorin domain (Kolodkin et al. (1993) "The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules." Cell *75*, 1389-1399). As noted above, plexins contain a more divergent but nevertheless conserved Semaphorin domain.

Semaphorins were originally characterized in the nervous system, where they have been implicated in repulsive axon guidance (Kolodkin et al. (1993) *supra*; Luo, Y., Raible, D., and Raper, J.A. (1993) "Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones." Cell *75*, 217-227; Tessier-Lavigne, M. and Goodman, C.S. (1996) "The molecular biology of axon guidance." Science *274*, 1123-1133). More recently, semaphorins have been furthermore implicated in cardiac and skeletal development (Behar, O., Golden, J.A., Mashimo, H., Schoen, F.J., and Fishman, M.C. (1996) "Semaphorin III is needed for normal patterning and growth of nerves, bones and heart." Nature *383*, 525-528), in the immune response (Hall, K.T., Boumsell, L., Schultze, J.L., Boussiotis, V.A., Dorfman, D.M., Cardoso, A.A., Bensussan, A., Nadler, L.M., and Freeman, G.J. (1996) "Human CD100, a novel leukocyte semaphorin that promotes B-cell aggregation and differentiation." Proc.Natl.Acad.Sci.U.S.A. *93*, 11780-11785), in the regulation of angiogenesis (Miao, H.Q., Soker, S., Feiner, L., Alonso, J.L., Raper, J.A., and Klagsbrun, M. (1999) "Neuropilin-1 mediates collapsin-1/Semaphorin III inhibition of endothelial cell motility. Functional competition of collapsin-1 and vascular endothelial growth factor-165" [In Process Citation]. J Cell Biol *146*, 233-242), and in tumor growth and metastasis (Christensen, C.R., Klingelhofer, J., Tarabykina, S., Hulgaard, E.F., Kramerov, D., and Lukanidin, E. (1998) "Transcription of a novel mouse semaphorin

gene, M-semaH, correlates with the metastatic ability of mouse tumor cell lines." Cancer Res. *58*, 1238-1244).

Previously identified plexins have been shown to be expressed in the developing nervous system, (i.e. Plexin-A is a receptor for class 1 semaphorins (Sema-1a and Sema-1b). Moreover, Plexin-A has been shown via genetic analysis to control motor and CNS axon guidance induced by semaphorins (Winberg, M.L., Noordermeer, J.N., Tamagnone, L., Comoglio, P.M., Spriggs, M.K., Tessier-Lavigne, M., and Goodman, C.S. (1998). Plexin A is a neuronal semaphorin receptor that controls axon guidance. Cell *95*, 903-916).

Thus a need exists for discovery of other members of the plexin family of proteins.

## SUMMARY OF THE INVENTION

The present invention provides four novel plexin family members.

In one aspect, the invention provides an isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of the amino acid sequence shown in (SEQ ID NO: 2), (SEQ ID NO: 4), (SEQ ID NO: 6) and (SEQ ID NO: 8).

In other aspects, the invention provides isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence selected from the group consisting of the nucleotide sequence shown in (SEQ ID NO: 1), (SEQ ID NO: 3), (SEQ ID NO: 5) and (SEQ ID NO: 7).

In another aspect, the invention provides a vector comprising the nucleic acid of the above-aspects.

The invention also provides an isolated polypeptide the amino acid sequence of which comprises residues 1-18, 19-518, 451-530, 601-680, 751-830, 800-1010, or 1196-1215 of SEQ ID NO: 2; 1-23, 24-507 or 1100-1119 of SEQ ID NO: 4; or 1-42, 43-600, 541-620, 691-770, 831-910, 900-1110 or 1270-1293 of SEQ ID NO: 6; or 8-49, 154-199 or 1-199 of SEQ ID NO: 8.

In another aspect, the invention provides an isolated polypeptide having at least 80% amino acid sequence identity to an amino acid sequence selected from the group consisting of the amino acid sequence shown in (SEQ ID NO: 2), (SEQ ID NO: 4), (SEQ ID NO: 6) and (SEQ ID NO: 8)

The invention also provides a chimeric molecule comprising a polypeptide of the above aspects fused to a heterologous amino acid sequence. In one embodiment the heterologous amino acid sequence is a Fc region of an immunoglobulin.

In other aspects, the invention provides an antibody that specifically binds to the polypeptides of the above aspects.

The invention also provides a method of treating, suppressing or altering a disorder involving aberrant immune regulation involving a signaling pathway between a plexin and a neuropilin in a mammal comprising the step of administering an effective amount of an agent to said mammal capable of interfering with the association between the plexin and neuropilin. Contemplated agents include a chimeric molecule or an antibody of the above aspects.

## DESCRIPTION OF THE DRAWINGS

**Figure 1.**

(A) Phylogenetic tree of human plexins. Known family members cluster in two major groups: plexin A and plexin-B subfamilies. (B) Structural features of plexins, Met-like receptors and semaphorins. In the extracellular moieties, yellow boxes indicate the "*sema*" domains and blue boxes mark the cysteine-rich MRS motifs, some of which are stippled to indicate their atypical sequence; atypical MRS are also found in the *sema domain* of semaphorins. Sequence identity among *sema* domains ranges from 15-50%, as previously described (see Winberg et al., 1998 *supra*). Potential furin-like proteolytic sites are marked by red ribbons. plexin-B1 "truncated" is the product of a splicing variant (see text). plexin-D1 and plexin-C1 (VESPR) are more distant family members, since they include atypical features in their extracellular domains. The intracellular domain of plexins (SP domain) is highly conserved through all family members, and it includes two separate regions of high homology (Maestrini, E., Tamagnone, T., Longati, P., Cremona, O., Gulisano, M., Bione, S., Tamanini, F., Neel, B.G., Toniolo, D., and Comoglio, P.M. (1996) "A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor." Proc. Natl. Acad. Sci. USA *93*, 674-678) (green oval and box). Met-like receptors are disulfide-bound heterodimers and include a cytoplasmic tyrosine kinase domain (red box). Mammalian semaphorins can be either secreted or cell surface proteins. Molecular weights of representative proteins are Plexin-A1 220 kDa, Plexin-B1 250 kDa, Plexin-C1 200

kDa, HGF-R/Met 145+45 kDa (heterodimer), Sema 4D 150 kDa (transmembrane), Sema7A approx. 100 kDa (membrane GPI-linked).

**Figure 2.**

**(a)** Cell surface semaphorins specifically bind human plexins. Micropraphs of the binding assays done testing i) the extracellular domain of semaphorin CD100 fused to alkaline phosphatase (CD100-AP) on COS cells transfected with *plexin-B1 cDNA*; ii) control AP on plexin-B1; iii) CD100-AP on plexin-B2; iv) CD100-AP on the entire extracellular domain of plexin-B1; v) CD100-AP on isolated "plexin-B1 truncated" (including *sema* domain, 1° and 2° MRS); vi) CD100-AP on a "plexin-B1-Δsema" (including 2° and 3° MRS; vii) extracellular domain of semaphorin A39R fused to AP, on plexin-C1 (Vespr); viii) SemaK1-AP on plexin-C1. The final detection of the binding was done either using alkaline phosphatase substrates (i-vi) or by immunofluorescence (vii and viii). **(B)** Scatchard analysis and binding curve of CD100-AP to plexin-B1 ($K_D = 0.9$ nM ± 0.15).

**Figure 3.**

Plexins associate with neuropilins *via* specific extracellular domains. Western blots of immunoprecipitated samples from cells co-expressing neuropilins and plexins. Specific MoAbs were used, directed against the VSV-tag included in plexins or the myc-tag included in neuropilin-2 (Np2, 130 kDa). Np2 co-immunoprecipitates with plexins, such as plexin-A3 (220 kDa), the extracellular domain of plexinA1 (approx.160 kDa), and plexin-B1 (250 kDa) but not with the unrelated cell surface receptor DCC (170 kDa). Np2 can also associate a truncated form of the extracellular moiety of plexin-B1 ("plex-B1 trunc.", approx. 110 kDa), containing the *sema domain.*

**Figure 4.**

Expression of mRNAs for plexins A1 (panel A, B), -A2 (panel C, D) and A3 (panel E, F) in the spinal cord (sc), dorsal root ganglia (d) and sympathetic ganglia (sg) of E13.5 mouse embryos. Expression of the mRNAs was detected by RNA in situ hybridization. Scale bar:1 μm.

**Figure 5.**

Effect of a truncated plexin-A1 construct (lacking the cytoplasmic domain) on repulsive and attractive responses of Xenopus spinal neurons to Sema3A and netrin-1. (A-F) A control spinal neuron exposed to a gradient of Sema3A emanating from a pipette (A) is repelled away over a period of 1 hr (B). In contrast, a GFP-expressing

spinal neuron from an embryo injected with mRNA for the truncated plexin-A1 construct (C) is not affected by Sema3A (D). A similar neuron (E) shows a normal attractive response to netrin-1 (F).

(G) Cumulative distribution plot of turning angles for all the neurons studied. Curves show the percent of neurons with turning angles less than the angle indicated on the abscissa, under different conditions (open circles, control neurons; black and blue circles, control neurons responding to Sema3A or netrin-1, respectively; red and green circles, responses of neurons expressing the truncated plexin-A1 construct to Sema3A and netrin-1, respectively. (H) Mean turning angle under all the conditions just mentioned.

**Figure 6.**

Tyrosine phosphorylation of plexin-A3 and plexin-B1. **(a)** Anti-phosphotyrosine western blotting of immunoprecipitated $p220^{plex\text{-}A3}$ and $p250^{plex\text{-}B1}$ proteins. plexin-B1 is larger since it contains an extra sequence between the second and the third MRS motif, in the extracellular domain (see Fig. 1). **(b)** The same immunoprecipitated samples underwent *in vitro* kinase assay in the presence of $[\gamma^{32}P]ATP$ , $Mg^{++}$ and $Mn^{++}$ ions. The SDS-PAGE was treated with alkali in conditions known to eliminate the phosphate labeling of Ser/Thr residues and specifically preserving phosphotyrosines.

**Figure 7**

Plexin-A3 overexpression mediates cell repelling cues. **(a)** Epithelial kidney MDCK cells transfected to overexpress plexin-A3 (or mock transfected) were cocultured with mesenchymal KJ-29 or NIH-3T3 cells. After 16-30 hours, mixed cultures of control cells (upper panels) reached confluency and stopped growing: typically the epithelial cells formed islets (circled) surrounded by a fibroblasts lawn. In contrast, MDCKs overexpressing plexin-A3 (lower panels) overwhelmed the adjacent mesenchymal cells. The latter withdrew and selectively detached from the culture dish (dying cell clusters are indicated by arrowheads), and eventually epithelial cells only survived. To allow an easier detection of the mesenchymal cells, these were labeled with DiI before being plated in mixed cultures. **(b)** Plexin-A3 expressing cells do not induce apoptotic signal on repelled fibroblasts. Mixed cultures of NIH 3T3 and control or plexin-A3 overexpressing MDCKs were tested for the presence of TUNEL-AP positive cells. Apoptotic cells were not present in clusters of repelled cells (indicated by

arrows). The right panel shows a positive control where apoptosis was induced on the same cells by UV treatment. (c) Plexin-A3 over-expressing cells form very transient contacts with fibroblasts. Time-lapse video-microscopy of control and plexin-A3 overexpressing MDCK cells grown in presence of fibroblasts. On top, snap-shot images from the movie, taken every 50 minutes (real time). In the upper row is shown the persistent contact of a fibroblast (marked by an arrow) with an islet of control MDCK cells (marked by a star). In the lower row another fibroblast, instead, forms a transient contact with an islet of plexin-A3 transfected cells, which also, in turn, reshapes. At the bottom, the diagrams show the relative frequency of persistent, transient or very-transient contacts between fibroblasts and MDCK cells.

## DETAILED DESCRIPTION OF THE INVENTION

The reference works, patents, patent applications, and scientific literature, including accession numbers to GenBank database sequences, that are referred to herein establish the knowledge of those with skill in the art and are hereby incorporated by reference in their entirety to the same extent as if each was specifically and individually indicated to be incorporated by reference. Any conflict between any reference cited herein and the specific teachings of this specification shall be resolved in favor of the later. Likewise, any conflict between an art-understood definition of a word or phrase and a definition of the word or phrase as specifically taught in this specification shall be resolved in favor of the latter.

Four novel human plexins have been identified: plexin-B2, plexin-B3, plexin-D1 and Plexin A-4. Plexin-A4 is located on human chromosome 7 and is a family member of the plexin-A subfamily which also includes plexin-A1 (alternatively named plexin-1/NOV), plexin-A2 (alternatively named plexin-2/OCT) and plexin-A3 (alternatively named plexin-2/SEX). Plexin-B2 and plexin-B3 are located on human chromosome 22 and chromosome X, respectively, and are family members of the plexin-B subfamily which also includes plexin-B1 (alternatively named SEP). Plexin-B3 maps very close to the plexin-A3 genomic locus on Xq28. Plexin-D1 is the first identified member of the plexin-D subfamily and is atypical of any of the other subfamilies. A fourth subfamily of plexins, the plexin-C subfamily, is defined by VESPR (now plexin-C1).

The four novel plexins as described herein have in their extracellular domains regions of homology with two other protein families: (a) Scatter Factors Receptors, encoded by the *MET* oncogene family (Tamagnone and Comoglio, 1997 *supra*), and (b)

Semaphorins (Kolodkin et al. (1993) *supra* (Figure 1b). In particular, plexins and Met-like receptors contain short cysteine-rich motifs, termed "Met Related Sequences" (MRS), whose minimal consensus is: C-X(5-6)-C-X(2)-C-X(6-8)-C-X(2)-C-X(3-5)-C (Maestrini et al., 1996 *supra*); Tamagnone and Comoglio, 1997 *supra*); blue boxes in Fig. 1B). The proteins of the Met family contain a single MRS (in their receptor β chains), whereas in plexin family members there are two/three repeated MRS motifs. Furthermore, all plexin family members contain in their extracellular moiety a 500 amino acid region similar to the sema domain of semaphorins (Kolodkin et al. (1993) *supra*; Winberg et al., 1998 *supra*); yellow boxes in Fig. 1B. The MRS motif is proposed to function as protein-protein-interaction-domain.

The cytoplasmic domain of plexins contains a ~600 amino acid domain which we term the SP domain ("Sex and Plexins", marked in green in Fig. 1B) that is highly conserved within the family (57-97% similarity) and in evolution (over 50% similarity between invertebrates and humans). The SP domain does not share homology with any known protein. It includes a number of potential tyrosine phosphorylation sites, but lacks the typical motifs of catalytic tyrosine kinases. Interestingly, the predicted secondary structure of the SP domain includes long conserved alpha helices, typically found in protein-protein interaction modules. Furthermore, there are several dihydrophobic amino acid motifs (such as LL or LI), known to mediate the internalization and downregulation of transmembrane receptors (Sandoval, I.V. and Bakke O. (1994). Targeting of membrane proteins to endosomes and lysosomes. Trends in Cell Biology *4*, 292-297).

The present invention also demonstrates that plexins can form complexes with neuropilins, which in turn demonstrates that a receptor for semaphorins can be hetero-oligomers of plexins and neuropilins. As demonstrated by in situ mRNA expression analysis, plexins and neuropilins are in fact simultaneously expressed in several neuronal populations during embryonic development. The plexin-neuropilin complex predates ligand binding, since the association is not influenced by the presence of class 3 semaphorins. That the observed plexin-neuropilin complexes are formed in *cis* is furthermore supported by the experimental conditions used (cotransfection of isolated cells with the two constructs). An interaction in *trans* might also be envisioned (considering that plexins and semaphorins share similar *sema* domains), however by

analyzing mixed cultures of cells separately transfected with plexins and neuropilins we did not isolate associated complexes (data not shown).

We observed that the main semaphorin binding domain of neuropilins (CUB domain (Giger, R.J., Urquhart, E.R., Gillespie, S.K., Levengood, D.V., Ginty, D.D., and Kolodkin, A.L. (1998) "Neuropilin-2 is a receptor for semaphorin IV: insight into the structural basis of receptor function and specificity." Neuron *21*, 1079-1092; Nakamura, F., Tanaka, M., Takahashi, T., Kalb, R.G., and Strittmatter, S.M. (1998) "Neuropilin-1 extracellular domains mediate semaphorin D/III-induced growth cone collapse" [In Process Citation]. Neuron *21*, 1093-1100; Chen et al. 1998 *supra*) is not required for the interaction with plexins, as indicated by the association of the relevant Neuropilin-2 deletion construct with plexin-B1 (not shown). A ternary complex, where neuropilins use two distinct protein modules to form a bridge between the sema domain of semaphorins and the sema domain of plexins is thus contemplated. It is further contemplated that plexins are the functional partners of neuropilins, required for transducing signals mediated by semaphorins, preferably class 3 semaphorins. For example, in flies, which lack both neuropilins and class 3 semaphorins, D Plex A appears sufficient as a functional receptor for Sema 1a, a transmembrane class 1 semaphorin (Winberg et al., 1998 *supra*). Further support that plexins are functional co-receptors for secreted semaphorins is demonstrated in an experiment that shows that a truncated plexin-A1 construct expressed in Xenopus spinal neurons abolishes repulsive responses to Sema3A without markedly affecting attractive responses to netrin-1. These results are consistent with the involvement of plexins.

The intracellular signals transduced by plexins are still largely obscure. The cytoplasmic domain of plexins is large and highly conserved within and across species and contains stretches of alpha helices, which are putative protein-protein interaction domains, and could thus mediate the association with cytosolic partners. We demonstrate herein that the cytoplasmic domain of plexins can be tyrosine phosphorylated, suggesting that, like other receptors devoid of intrinsic catalytic activity, plexins may signal by associating a tyrosine kinase (Stahl, N. and Yancopoulos, G.D. (1993). The alphas, betas, and kinases of cytokine receptor complexes. Cell *74*, 587-590; Glass, D.J., Bowen, D.C., Stitt, T.N., Radziejewski, C., Bruno, J., Ryan, T.E., Gies, D.R., Shah, S., Mattsson, K., Burden, S.J., DiStefano, P.S.,

Valenzuela, D.M., DeChiara, T.M., and Yancopoulos, G.D. (1996). Agrin acts via a MuSK receptor complex. Cell *85*, 513-523) .

In addition, we show herein that expression of plexins, particularly plexin-A3, mediates cell-repelling cues. By time-lapse video-microscopy we observed a true repelling effect on fibroblasts. Intriguingly, we observed that -upon interaction with fibroblasts- also the islets of plexin-A3 MDCKs at times reshaped. This may be explained by the existence of intra-epithelial repelling cues, balanced by the attractive forces exerted by epithelial cell junctions.

Moreover we have demonstrated that in the nervous system (i.e. Drosophila), that defasciculating motor axons co-express both Plexin A and one of its interacting partners, the transmembrane semaphorin Sema-1a (Winberg et al., 1998 *supra*). This demonstrates that plexins act *in vivo* either as receptors or ligands for cell surface semaphorins, which in turn can transduce intracellular signals, as reported for ephrins (Holland et al., 1996 *supra*). Semaphorins, therefore, besides being pivotal in axon guidance, have a general role in morphogenesis and tissue remodeling by mediating cell-repelling cues via their interactions with plexins.

Accordingly, in a first aspect, the invention provides an isolated nucleic acid molecule encoding a novel human plexin polypeptide. By "plexin polypeptide" is meant a member of the plexin family comprising an amino acid sequence that shares at least 60% amino acid sequence homology with SEQ ID NOS: 2 (plexin B-2), 4 (plexin B-3), 6 (plexin D-1) or 8 (plexin A-4), preferably, at least 65% sequence homology, more preferably, at least 70% sequence homology, more preferably, at least at least 75% sequence homology, more preferably, at least 80% sequence homology, still more preferably at least 85% sequence homology, even more preferably, at least 90% sequence homology, and most preferably at least 95% sequence homology with SEQ ID NOS: 2, 4, 6 or 8. Plexin polypeptides of the invention are useful for modulating cell growth (i.e. nerve) and immune regulation.

As used herein, by "modulating" is meant increasing or decreasing cell growth. By "cell growth" is meant any change in cell number or size, including, without limitation, increase or decrease in cell number, increase or decrease in rate of cell division, increase or decrease in rate of cell death, and/or increase or decrease in cell size. Standard methods for measuring cell growth include standard apoptosis assays (*e.g.*, TUNEL assays, DNA fragmentation, trypan blue exclusion) and cell proliferation assays

(*e.g.*, $^{3}$H-thymidine incorporation). It will be appreciated that the degree of modulation of cell growth provided by a plexin polypeptide in a given assay will vary, but one of skill in the art can readily determine the statistically significant change in cell growth of a cell exposed to a plexin polypeptide.

By "immune regulation" is meant increasing or decreasing the biological functions of immune cells (*i.e.*, cells involved in an immune response). Immune cells include, without limitation, lymphocytes (T and B), NK cells, dendritic cells, myeloid cells (*e.g.*, macrophages and neutrophils), and other hematopoietic cells involved in an immune response.

By "nucleic acid molecule" or "nucleic acid" as used herein, is meant any deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), including, without limitation, complementary DNA (cDNA), genomic DNA, RNA, heteronuclear RNA (hnRNA), messenger RNA (mRNA), DNA/RNA hybrids, or synthetic nucleic acids (*e.g.*, an oligonucleotide) comprising ribonucleic and/or deoxyribonucleic acids or synthetic variants thereof. The nucleic acid of the invention includes, without limitation, an oligonucleotide or a polynucleotide. The nucleic acid can be single stranded, or partially or completely double stranded (duplex). Duplex nucleic acids can be homoduplex or heteroduplex.

By "polypeptide" is meant any molecule comprising two or more amino acids joined together with a peptide bone, regardless of length or post-translational modifications (*e.g.*, without limitation, glycosylation, lipidation, acetylation, or phosphorylation). Useful plexin polypeptides of the invention include, without limitation, the full length plexin polypeptides having the amino acid sequence of SEQ ID NOS: 2, 4 , 6, 8 or 10,; an extracellular domain of the polypeptide having the amino acid sequence 1 to 1199 of SEQ ID NO: 2; 1 to 1099 of SEQ ID NO: 4; 1 to 1270 of SEQ ID NO: 6 or 1 to 199 of SEQ ID NO: 8, with its associated signal peptide; or an extracellular domain of the polypeptide having the amino acid sequence 19 to 1199 of SEQ ID NO: 2; 24 to 1099 of SEQ ID NO: 4; or 43 to 1270 of SEQ ID NO: 6, lacking its associated signal peptide; an intracellular domain of the polypeptide having the amino acid sequence of SEQ ID NOS: 2, 4, 6 or 8; and polypeptides, the amino acid sequence of which comprises about residues 1-18 (putative signal sequence), 19-518 (sema domain), 451-530 (1° MRS), 601-680 (2° MRS), 751-830 (3° MRS), 800-1010 (G-P repeats) or 1196-1215 (putative transmembrane domain) of SEQ ID

NO: 2; about residues 1-23 (putative signal sequence), 24-507 518 (sema domain) or 1100-1119 (putative transmembrane domain) of SEQ ID NO: 4; or about residues 1-42 (putative signal sequence), 43-600 (sema domain), 541-620 (1° MRS), 691-770 (2° MRS), 831-910 (3° MRS), 900-1110 (G-P repeats) or 1270-1293 (putative transmembrane domain) of SEQ ID NO: 6; or about residue 8-49 (1° MRS) or 154-199 (2° MRS) of SEQ ID NO: 8.

By "isolated" is meant a compound (*e.g.*, a nucleic acid molecule or a protein) that has been separated from components (*e.g.*, nucleic acid molecules, proteins, lipids, and/or carbohydrates) which naturally accompany it. Water, buffers, and other small molecules (*e.g.*, molecules having a molecular weight of less than about 1000 daltons) may accompany an isolated compound of the invention. Preferably, an isolated compound is at least 70%, by weight, free from components which naturally accompany it. More preferably, an isolated is at least 75%, by weight, free from components which naturally accompany it; still more preferably, at least 80%, by weight, free; even more preferably, at least 85%, by weight, free; and even more preferably, at least 90%, by weight, free from components which naturally accompany it. Most preferably, a substantially purified compound is at least 95%, by weight, free from components which naturally accompany it.

Where the isolated compound is a nucleic acid molecule, the isolated nucleic acid molecule is separated from other nucleic acids (*e.g.*, genes or transcripts) or proteins which, in the naturally-occurring genome of the organism from which the nucleic acid molecule was derived, flanked the nucleic acid molecule. Isolated nucleic acid molecules therefore include, without limitation, a recombinant nucleic acid molecule incorporated into a plasmid or other vector (*e.g.*, a replication-defective virus); a recombinant nucleic acid molecule incorporated into the genome of a prokaryotic or eukaryotic organism; or a nucleic acid molecule which exists as a separate molecule independent of other nucleic acids (*e.g.*, a PCR product, a chemically synthesized nucleic acid molecule, or a nucleic acid molecule produced by restriction endonuclease digestion). Purification of a nucleic acid molecule can be accomplished and measured by any standard method including, without limitation, sequence analysis, chemical synthesis, PCR, CsCl gradient, phenol:chloroform extraction, ethanol precipitation, Southern or Northern blotting analysis followed by band extraction and purification, and

high performance liquid chromatography (HPLC; see, *e.g.*, Fisher (1980) Laboratory Techniques in Biochemistry and Molecular Biology, Work and Burdon (eds.), Elsevier).

Thus, in one non-limiting example, to obtain an isolated nucleic acid molecule encoding a plexin polypeptide, a nucleic acid molecule is chemically synthesized on a standard oligonucleotide synthesis machine. The resulting single stranded molecule is then subjected to second strand synthesis to form a duplex DNA molecule, which is then ligated into a plasmid capable of replication in a prokaryotic or eukaryotic cell. The nucleic acid molecule is then replicated in the cell, purified (*e.g.*, by CsCl gradient), and subjected to sequence analysis.

In certain embodiments of the first aspect of the invention, the nucleic acid molecule has a nucleic acid sequence comprising SEQ ID NOS: 1, 3, 5, 7 or 9. Preferably, the nucleic acid molecule of the invention has not more than 500 nucleotides flanking each of the 5' and 3' ends of SEQ ID NOS: 1, 3, 5, 7 or 7. In certain embodiments, the plexin polypeptide has an amino acid sequence that comprises SEQ ID NOS: 2, 4, 6, 8 or 10. Preferably, the plexin polypeptide of the invention has not more than 50 amino acid residues flanking each of the N-terminal and C-terminal ends of SEQ ID NOS: 2, 4, 6, 8 or 10.

In certain embodiments of the first aspect of the invention, the nucleic acid molecule hybridizes under stringent conditions (as defined herein) to SEQ ID NOS: 1, 3, 5, 7 or 9.

The invention also includes nucleic acid molecules that hybridize under stringent hybridization conditions (as defined herein) to all or a portion of the nucleotide sequence represented by SEQ ID NOS: 1, 3, 5, 7 or 9 or its complement. The hybridizing portion of the hybridizing nucleic is at least 80%, *e.g.*, at least 95%, or at least 98%, homologous to the sequence of a portion or all of a nucleic acid encoding a polypeptide having the amino acid sequence of SEQ ID NOS: 2, 4, 6, 8 or 10, or its complement. Hybridizing nucleic acids of the type described herein can be used, for example , as a cloning probe, a primer (*e.g.*, a PCR primer) or a diagnostic probe.

Hybridization of the oligonucleotide probe to a nucleic acid sample typically is performed under stringent conditions. Nucleic acid duplex or hybrid stability is expressed as the melting temperature or Tm, which is the temperature at which a probe dissociates from a target DNA. This melting temperature is used to define the required stringency conditions. If sequences are to be identified that are related and substantially

identical, rather than identical, then it is useful to first establish the lowest temperature at which only homologous hybridization occurs with a particular concentration of salt (*e.g.*, SSC or SSPE). Then, assuming that 1% mismatch results in a 1°C decrease in the Tm, the temperature of the final wash in the hybridization reaction is reduced accordingly (for example, if the sequences have > 95% identity with the probe are sought, the final wash temperature is decreased 5°C). In practice, the change in the Tm can be between 0.5 C and 1.5 C per 1% mismatch. "Stringent conditions" involve hybridization at 68°C in 5x SSC/5x Denhardt's solution/1.0% SDS, and washing in 0.2xSSC/0.1% SDS at room temperature. "Moderately stringent conditions" include washing in 3xSSC at 42°C. The parameters of salt concentration and temperature can be varied to achieve the optimal level of identity between the probe and the target nucleic acid. Additional guidance regarding such conditions is readily available in the art, for example, by Sambrook *et al., supra*; and Ausubel *et al., supra.*

Nucleic acid sequence homology (as well as amino acid sequence homology) can be measured according to standard methods. Unless otherwise specified, as used herein used herein, "percent homology" of two amino acid sequences or of two nucleic acids is determined using the algorithm of Karlin and Altshul (*Proc. Natl. Acad. Sci. USA* **87**: 2264-2268, 1990), modified as in Karlin and Altschul (*Proc. Natl. Acad. Sci. USA* **90**: 5873-5877, 1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (*J. Mol. Biol.* **215**: 403-410, 1990). BLAST nucleotide searches are performed with the NBLAST program, e (score) = 100, word length = 12, to obtain nucleotide sequences homologous to a nucleic acid molecule of the invention. BLAST protein searches are performed with the XBLAST program, e (score) = 50, word length = 3, to obtain amino acid sequences homologous to a reference polypeptide (*e.g.*, SEQ ID NO: 2). To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul et al. (*Nucleic Acids Res.* **25**: 3389-3402, 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) are used, namely e=10; w=11 for nucleic acid; w=3 for amino acid (the Blosum 62 scoring matrix); low complexity sequence filtering. The default settings of BLAST emphasize regions of local alignment to detect relationships among sequences which share only isolated regions of similarity (Altschul et al., *J. Mol. Biol.* **215**: 403-410 (1990). See http://www.ncbi.nlm.nih.gov.

Thus, in a non-limiting example to obtain an isolated nucleic acid molecule encoding a plexin polypeptide, a nucleic acid molecule having the sequence of SEQ ID NOS: 1, 3, 5 or 7 is used to probe a cDNA library under stringent conditions according to standard techniques (see., *e.g.*, Ausubel *et al.*, *supra*). Upon identification of a positive clone (*i.e.*, a clone that hybridizes to SEQ ID NOS: 1, 3, 5 or 7 under stringent conditions), that clone is expanded and subjected to sequence analysis. A nucleic acid molecule having a nucleic acid sequence that is at least 70% identical, preferably at least 75% identical, more preferably, at least 80% identical, still more preferably at least 85% identical, even more preferably, at least 90% identical, and most preferably at least 95% identical (as measured by the basic BLAST program of NCBI on default settings) to SEQ ID NOS: 1, 3, 5 or 7 is a nucleic acid molecule of the invention.

In a second aspect, the invention provides four novel isolated plexin polypeptides.

"Isolated" is as defined for the first aspect of the invention. Where the isolated compound is a polypeptide, the isolated polypeptide is separated from organic molecules, such as nucleic acid molecules, polypeptides, and/or carbohydrates, which, in the naturally-occurring organism from which the polypeptide was derived, accompany the polypeptide. Isolated polypeptides therefore also include a recombinant polypeptide (*e.g.*, a human polypeptide expressed in an insect cell), or a chemically synthesized polypeptide. Purification of a polypeptide can be accomplished and measured by any standard method including, without limitation, chemical synthesis, recombinant polypeptide expression in prokaryotic or eukaryotic cells, affinity chromatography, Western blotting analysis, SDS-PAGE analysis, and/or HPLC.

In accordance with this aspect, the invention provides all derivatives, mutants, truncations, and/or splice variants of the four novel plexin polypeptides, so long as these derivatives, mutants, truncations, and/or splice variants share at least 60% amino acid sequence homology with SEQ ID NOS: 2 ,4 ,6 or 8, preferably, at least 65% sequence homology, more preferably, at least 70% sequence homology, more preferably, at least at least 75% sequence homology, more preferably, at least 80% sequence homology, still more preferably at least 85% sequence homology, even more preferably, at least 90% sequence homology, and most preferably at least 95% sequence homology with SEQ ID NOS: 2 ,4 ,6 or 8 as determined using the basic BLAST program of the National Center for Biotechnology (NCBI; National Library of Medicine, Bethesda, MD), using the

default settings defined therein using the sequence of the four novel plexin derivative, mutant, truncation and/or splice variance as the probe.

Preferred plexin polypeptide derivatives include polypeptides whose sequences differ from the sequence given in SEQ ID NOS: 2 ,4 ,6 or 8, by one or more conservative amino acid substitutions, or by one or more non-conservative amino acid substitutions, deletions or insertions which do not abolish the biological activity of the plexins. Conservative amino acid substitutions typically include the substitution of one amino acid for another with similar biochemical characteristics, such as polarity, size, and/or charge. Non-limiting examples of conservative substitutions are substitutions within the following groups: valine, glycine, glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine, phenylalanine, and tyrosine.

Useful methods for mutagenesis to generate plexin mutants are known in the art (see, *e.g.*, Sambrook *et al., supra*; Ausubel *et al., supra*). Preferred methods for mutagenesis are described in PCT Publication WO99/12965 and include PCR mutagenesis and saturation mutagenesis. A library of random amino acid sequence variants can also be generated by the synthesis of a set of degenerate oligonucleotide sequences.

In certain embodiments of the second aspect of the invention, the plexin polypeptide has a sequence comprising the sequence of SEQ ID NOS: 2 ,4 ,6 or 8. In one non-limiting example, in accordance with the invention, an isolated plexin polypeptide comprising the sequence of SEQ ID NOS: 2 ,4 ,6 or 8 can chemically synthesized according to standard techniques (*e.g.*, at a commercial peptide generating facility).

For example, a putative plexin polypeptide is purified and subjected to N-terminal sequencing to determine its amino acid sequence. The amino acid sequence of the polypeptide is then compared to SEQ ID NOS: 2 ,4 ,6, 8 or 10 (as measured by the basic BLAST program of NCBI on default settings). A polypeptide that shares at least 60% homology with SEQ ID NOS: 2 ,4 ,6, 8 or 10 is a plexin polypeptide of the invention.

In another example, purification of a plexin polypeptide is facilitated by the addition of a tag to the polypeptide that enables purification of the tagged polypeptide. Non-limiting examples of a tag include a hemagglutinin (HA) tag, a his tag, a GST tag, a

FLAG-tag, and a myc tag. Thus, a nucleic acid molecule of the first aspect is engineered using standard molecular biology techniques to incorporate the nucleic acid sequence encoding the tag. The engineered nucleic acid molecule is then introduced and positioned for expression in an appropriate cell to produce the recombinant tagged polypeptide, which can then be readily purified by binding of the tag to its substrate. For example, the his tag binds to Ni-NTA agarose. Likewise, a GST (glutathione S-transferase) tag binds to glutathione agarose beads. Both his tag and GST tag expression and purification kits are commercially available from PharMingen (San Diego, CA). Likewise, myc-tagged plexin polypeptide are produced by cells introduced with a nucleic acid molecule encoding the tagged protein and positioned for expression in the cell.

It will be appreciated that particularly useful polypeptides of this aspect of the invention are secreted by the cell in which they are produced, thus facilitating purification of the polypeptide from the culture media in which the cells have been maintained, without requiring lysis of the cell.

In a third aspect, the invention provides a cell engineered to comprise a nucleic acid molecule encoding one of the four plexin polypeptides. By "engineered" is meant that the cell of the invention has been modified by standard molecular biology techniques. Where the cell is "engineered to comprise a nucleic acid molecule," standard molecular biology techniques have been employed to introduce the indicated nucleic acid molecule into the cell, either by transformation or transfection of the cell with a plasmid, or by infection or transduction of the cell with a recombinant virus.

The nucleic acid molecule of the first aspect of the invention is preferably subcloned into a plasmid or vector (for example, but not limited to, a vector used to generate a recombinant virus), wherein the nucleic acid molecule is positioned for expression in the plasmid or vector. The plasmid or vector is then introduced into a cell by standard techniques to produce an engineered cell in accordance with the third aspect of the invention.

In certain embodiments of the third aspect, the cell is a prokaryotic cell (*e.g.*, a bacterium). For example, *E. coli* cells (*e.g.*, DH5α) are transformed (using, *e.g.*, electroporation) with a bacterial plasmid (*i.e.*, a plasmid containing an *E. coli* origin of replication) containing a nucleic acid molecule of the first aspect of the invention. The transformed bacteria are selected using, for example, an antibiotic-resistance encoding nucleic acid molecule (*e.g.*, ampicillin resistance) on the plasmid such that the antibiotic

resistance protein is expressed by the transformed bacteria. The transformed bacteria are then propagated, and can be cryopreserved and stored frozen in glycerol.

Those of skill in the art will appreciate that in accordance with the third aspect of the invention, a nucleic acid molecule encoding one of the four plexin polypeptides may be introduced into a large variety of cells. For example, a nucleic acid molecule encoding one of the four plexin polypeptides can be introduced into prokayotic cells (*e.g.*, bacteria), and any eukaryotic cell into which an exogenous nucleic acid molecule may be introduced. Thus, in certain embodiments of the third aspect of the invention, the cell is a eukaryotic cell. Eukaryotic cells according to this aspect of the invention that comprise a nucleic acid molecule encoding one of the four plexin polypeptides include, without limitation, yeast cells, plant cells, insect cells, and mammalian cells. Within the category of mammalian cells are cells from any mammalian species (including, without limitation, mouse, hamster, monkey, human), of any lineage (including, without limitation, lymphocyte, fibroblast, stem cell), and may be an immortalized cell, or a non-immortalized cell. Cells, as well as plasmids and/or vectors (*e.g.*, vectors that can be packaged to form infectious virus particles), are commercially available, for example, from the American Type Culture Collection ("ATCC"; Manassas, VA).

In certain embodiments of the third aspect of the invention, the nucleic acid molecule is positioned for expression in the cell. By "positioned for expression" is meant that the nucleic acid molecule is operably linked to at least one regulatory sequence which directs the transcription and translation of the nucleic acid molecule in a cell, such that the cell engineered to comprise the nucleic acid molecule produces (*i.e.*, expresses) the protein encoded by the nucleic acid molecule. By "operably linked" is meant that the nucleic acid molecule and the regulatory sequence are connected in a such a way as to permit expression of the nucleic acid molecule when the nucleic acid molecule is present in a cell. Regulatory sequences include, without limitation, promoters, enhancers, IRES sequences, and polyadenylation signals. Since plexin polypeptides are involved in immune regulation and the modulation of cell growth, it may be desirable to operably link a nucleic acid molecule encoding one of the four plexin polypeptides to an inducible promoter.

The four plexin polypeptides that are encoded by the nucleic acid molecules do not necessarily include the transmembrane domain of the four plexin polypeptides, and so may be produced by the cell as an intracellular polypeptide or a soluble secreted

polypeptide. For example, if the polypeptide fragment is secreted by the cell, it can be purified from the conditioned growth media of the transfected cells, without having to lyse the cells. Likewise, although a soluble intracellular polypeptide fragment is purified from only lysed cells, the fragment, being soluble, does not have to be extracted from the cell membrane; thus, different lysis conditions may be used to obtain purified soluble intracellular polypeptide fragment as compared to the lysis conditions required to obtain purified full length plexin polypeptides (which has a transmembrane domain).

Protein expression systems have been established for a variety of cells and are known to those of skill in the art. Cells are also commercially available from the ATCC, and a variety of protein expression system kits are commercially available from, for example, Invitrogen Corp. (Carlsbad, CA), Clontech Laboratories (Palo Alto, CA), PharMingen (San Diego, CA), Promega Corp. (Madison, WI), and Stratagene (La Jolla, CA).

For example, a nucleic acid molecule encoding one of the four plexin polypeptides is operably linked to bacterial regulatory sequences (*e.g.*, T7 late promoter or bacteriophage regulatory sequences), and the resulting nucleic acid molecule is used to transform bacterial cells, where the transformed bacterial cells produce one of the four plexin polypeptides. In another example, a nucleic acid molecule encoding one of the four plexin polypeptides is operably linked to baculovirus regulatory sequences in a baculovirus vector. Recombinant baculovirus are then generated and used to transduce insect cells (using, for example, the expression kit commercially available from Clontech Laboratories. The transduced insect cells comprise a nucleic acid molecule encoding one of the four plexin polypeptides positioned for expression in the insect cell, and thus produce one of the four plexin polypeptides.

Mammalian cells are widely used as protein expression systems. For example, a mammalian cell may be transduced with a recombinant retrovirus or adenovirus comprising a nucleic acid molecule encoding one of the four plexin polypeptides operably linked to regulatory sequences that are either endogenous to the particular virus or exogenous to the virus (*e.g.*, a CMV promoter in a retroviral vector). The transduced mammalian cell is then propagated *in vitro* in tissue culture, *in vivo* (*e.g.*, in an immunocompromised animal), and/or cryopreserved and stored frozen in DMSO.

In another example, mammalian cells are transfected with an expression plasmid comprising a nucleic acid molecule encoding one of the four plexin polypeptides

operably linked to one or more regulatory sequences on the plasmid. By "expression plasmid" is meant a plasmid in which an inserted nucleic acid molecule of interest (*e.g.*, encoding one of the four plexin polypeptides, a plexin chimeric molecule, or tagged plexin polypeptide) is operably linked to at least one regulatory sequence such that when the expression plasmid containing the inserted nucleic acid molecule of interest is introduced (*e.g.*, by transfection) into a cell, the inserted nucleic acid molecule is positioned for expression in that cell. The nucleic acid molecule in the expression plasmid, upon being introduced into the cell, is thus positioned for expression in that cell, and enables the cell to produce one of the four plexin polypeptides encoded by the nucleic acid molecule.

In one non-limiting example, a nucleic acid molecule according to the first aspect of the invention is inserted into a standard mammalian expression plasmid (*e.g.*, pcDNA3.1 commercially available from Invitrogen Corp., Carlsbad, California), such that the inserted nucleic acid molecule encoding one of the four plexin polypeptides is operably linked to the regulatory sequences in the mammalian expression plasmid. Mammalian cells are then transfected with this expression plasmid (using, *e.g.*, $CaPO_4$ or DEAE-dextran). Where the expression plasmid contains an antibiotic-resistance encoding nucleic acid molecule (*e.g.*, neomycin resistance on the pCDNA3.1 plasmid) such that the antibiotic resistance protein is expressed by the transfected cells, transfected cells may be selected for the ability to grow in the presence of the antibiotic. The transfected cells may then be propagated and cryopreserved and stored in frozen in DMSO.

In a fourth aspect, the invention provides an isolated nucleic acid molecule encoding a chimeric molecule comprising at least two segments, wherein one of the segments comprises one of the four plexin polypeptides. By "chimeric molecule" is meant a protein that comprises at least two segments of polypeptide joined together by any means, including, without limitation, a covalent bond (*e.g.*, peptide bond), a non-covalent bond (*e.g.*, ionic bond or hydrogen bond) or by a chemical crosslinker. It should be noted that one of the four plexin polypeptides that has been tagged is within the definition of a chimeric molecule.

In certain embodiments of the fourth aspect of the invention, the nucleic acid molecule encoding the segment of a chimeric molecule comprising one of the four plexin

polypeptides hybridizes under stringent conditions to SEQ ID NO: 1, 3, 5 or 7. "Stringent conditions" are as described above for the first aspect of the invention.

Standard molecular biology techniques may be employed to generate nucleic acid molecules encoding chimeric molecules according to the fourth aspect of the invention. For example, a nucleic acid molecule encoding the extracellular domain of one of the four plexin polypeptides may be joined, in frame, to a nucleic acid molecule encoding the constant region of an immunoglobulin molecule (see, *e.g.*, Chamow S.M., Antibody Fusion Proteins, John Wiley & Sons, New York, 1999). By "in frame" is meant that a first nucleic acid molecule is ligated to a second nucleic acid molecule such that the each of the amino acid sequences of the polypeptides encoded by each of the first and the second nucleic acid molecules is not frame-shifted.

In one non-limiting example, a chimeric molecule comprising the extracellular domain of one of the four plexin polypeptides including the amino acid sequence of SEQ ID NOS: 2, 4, 6 or 8 is generated. In this example, a nucleic acid molecule encodes amino acid residue number 1(19) through about amino acid residue number 1199 of SEQ ID NO: 2; amino acid residue number 1(24) through about amino acid residue number 1099 of SEQ ID NO: 4; amino acid residue number 1(43) through about amino acid residue number 1270 of SEQ ID NO: 6 and amino acid residue number 1 through about amino acid residue number 199 of SEQ ID NO: 8 with its associated signal peptide (parenthesis depicts about the beginning of the amino acid sequence of the extracellular domain lacking its signal peptide). This nucleic acid molecule is fused in frame with a nucleic acid molecule encoding the constant region of an immunoglobulin, such that the chimeric molecule encoded by the resulting nucleic acid molecule generally has the following structure:

| | | | |
|---|---|---|---|
| N-terminus | extracellular domain of SEQ ID NO: 2 with or lacking its signal peptide | amino acids from the constant region of an Ig molecule | C-terminus |
| N-terminus | extracellular domain of SEQ ID NO: 4 with or lacking its signal peptide | amino acids from the constant region of an Ig molecule | C-terminus |
| N-terminus | extracellular domain of SEQ ID NO: 6 with or lacking its signal peptide | amino acids from the constant region of an Ig molecule | C-terminus |
| N-terminus | extracellular domain of SEQ ID NO: 8 with or lacking its signal | amino acids from the constant region of an Ig molecule | C-terminus |

| | | | |
|---|---|---|---|
| | peptide | | |

The heavy chain class (*e.g.*, IgG, IgA, IgM, IgD, or IgE) can be varied in this chimeric molecule depending upon which constant region is used. Nucleic acid molecules encoding the constant region of various immunoglobulin (Ig) heavy chains are known (see, *e.g.*, Zettlmeissl et al., *DNA Cell Biol.* **9(5)**:347-53, 1990) Indeed, expression plasmids are available, into which the nucleic acid molecule of interest (*i.e.*, a nucleic acid molecule encoding an extracellular domain of the polypeptide of SEQ ID NO: 2; SEQ ID NO: 4; SEQ ID NO: 6; or SEQ ID NO: 8) can be inserted, and the resulting plasmid introduced into a cell to produce one of the four extracellular plexin-Ig chimeric molecule s (see, *e.g.*, Zettlmeissl et al., *supra*; Miller et al., *J. Exp. Med.* **178 (1)**: 211-222, 1993).

Any variety of chimeric molecule carrying the extracellular domains of one of the four plexin polypeptide may be generated. For example, the extracellular domain of one of the four plexin polypeptides can be myc-tagged, his-tagged, or FLAG tagged according to standard molecular biology techniques.

Such extracellular proteins are particularly useful for identifying ligands to which the extracellular domain of one of the four plexin polypeptides bind. For example, extracellular plexin-D1-Ig chimera can be immobilized on a protein A-sepharose column. Molecules suspected of binding the extracellular domain of plexin-D1 are added to the column, to which the molecule that binds to the extracellular domain of plexin-D1 adhere, and the non-binding molecules flow through the column. The extracellular plexin-D1-binding molecules are readily eluted, for example, by changing the pH or ion concentration of the elution buffer.

Extracellular plexin proteins are also used to identify cells expressing the ligand of plexin extracellular domain on their cell surface (and thereby also identify the ligand itself). For example, cells are incubated with a FLAG-tagged plexin extracellular domain chimeric molecule. A FLAG-tagged plexin extracellular domain chimeric molecule is generated. An anti-FLAG antibody that is detectably labeled is then added to the cells. By "detectably labeled" is meant any means for marking and identifying the presence of a molecule. Detectable labels include, without limitation, radioactive labels (*e.g.*, $^{32}P$ or $^{35}S$) and fluorophore labels (*e.g.*, FITC, phycoerythrin, or rhodamine). For example, FITC-labeled anti-FLAG antibodies are commercially available from Babco, Richmond,

CA. The "stained" cells (*i.e.*, cells incubated first with the FLAG-tagged plexin extracellular domain chimeric molecule and then with the FITC-labeled anti-FLAG antibody), are then subjected to flow cytometry analysis to select those cells that are labeled with FITC, and so express a molecule that binds to the extracellular domain of one of the four plexin polypeptides. The FITC labeled cells are then further manipulated (*e.g.*, characterized to determine which cells express the plexin polypeptide ligand).

The ligand of the plexin extracellular domain is itself identified, for example, by lysing the cells, adding the lysate to one of the four plexin extracellular domain-Ig chimeric molecule columns described above, and purifying the ligand. The ligand is then sequenced by N-terminal sequencing.

In another non-limiting example, the intracellular domain of one of the four plexin polypeptides is used as "bait" in a yeast two-hybrid system to identify ligands that interact with the intracellular domain of one of the four plexins described herein. For general description of the two-hybrid system, see U.S. Patent Nos. 5,283,173; 5,468,614; and 5,695,941. In this example, a nucleic acid molecule encoding from about amino acid residue number 143 through at least amino acid residue number 214 of SEQ ID NO: 2 is inserted into a standard DNA binding domain expression plasmid (*e.g.*, the GAL4 DNA binding domain plasmid in the Interactor kit commercially available from PharMingen (San Diego, CA). (It will be understood that the nucleic molecule may encode amino acid residue number 138-148 through at least amino acid residue number 214 of SEQ ID NO: 2.) A variety of cDNA libraries in transcriptional activation domain vectors are available (*e.g.*, from Clontech, Palo Alto CA). The cDNA libraries are screened employing standard methods (see, *e.g.*, the methods employed in U.S. Patent No. 5,780,262) to identify cDNA clones encoding a ligand that binds to the intracellular domain of one of the four plexin polypeptides. One preferable cDNA library screened in this example is a cDNA library generated from an immune cell (*e.g.*, a lymphocyte or NK cell).

In a fifth aspect, the invention provides a purified chimeric molecule comprising one of the four plexin polypeptides. Methods for purifying proteins are as described for the second aspect of the invention.

In a sixth aspect, the invention provides a cell engineered to comprise a nucleic acid molecule encoding a chimeric molecule comprising at least two segments, wherein one of the segments comprises one of the four plexin polypeptides. As described for the

third aspect of the invention, a nucleic acid encoding a chimeric molecule comprising one of the four plexin polypeptides may be introduced into any variety of cells. In certain embodiments, the cell is a prokaryotic cell or a eukaryotic cell. In certain embodiments, the eukaryotic cell is a yeast cell or a mammalian cell (*e.g.*, a human cell).

In a seventh aspect, the invention provides an isolated binding agent that specifically binds one of the four plexin polypeptides, or specifically binds a chimeric molecule comprising a segment comprising one of the four plexin polypeptides. In certain embodiments, the plexin protein has an amino acid sequence comprising SEQ ID NOS:2, 4, 6 or 8.

By "specifically binds" is meant a binding agent (*e.g.*, an antibody) that binds to its specific target (*e.g.*, one of the four plexin polypeptides) with greater affinity than it binds to other molecules. Preferably, where the binding agent is an antibody, the antibody preferably specifically binds to its specific target with a dissociation constant ($K_D$) of at least $10^{-5}$ M, more preferably, $10^{-6}$ M, even more preferably $10^{-7}$ M, and most preferably, the binding agent specifically binds to its specific target with a $K_D$ of at least $10^{-8}$ M.

Preferably, the binding agent of this aspect of the invention is an antibody, such as a monoclonal antibody or a polyclonal antibody, or a fragment of an antibody that specifically binds one of the four plexin polypeptides. Standard methods may be employed to generated both monoclonal and polyclonal antibodies that specifically bind to one of the four plexin polypeptides of the invention. See, *e.g.*, Ausubel et al., *supra*; Coligan, J.E. et al., Current Protocols in Immunology, John Wiley & Sons, New York (1991); and Delves, P.J., Antibody Production: Essential Techniques, John Wiley & Sons, New York (1997). Briefly, the plexin polypeptides of the present invention, purified according to the methods described for the second aspect of the invention, are used to immunize rabbits (*e.g.*, for polyclonal antibodies) or mice (*e.g.*, for monoclonal antibodies) to generate antibody-mediated immunity to the four plexin polypeptides used to immunize the animal. For monoclonal antibodies, antibodies can be screened by, *e.g.*, ELISA, to identify those antibodies that show the highest affinity for the immunizing plexin protein of polypeptide fragment. The cloned cell producing the high affinity monoclonal antibody can then propagated *in vitro* (where the antibody is purified from the culture supernatant) or *in vivo* (where the antibody is purified from ascites fluid), and

can also be cryopreserved and stored frozen at, *e.g.*, -70°C in DMSO, to provide a potentially limitless supply of monoclonal antibody.

In addition to intact monoclonal and polyclonal antibodies, the invention also provides various antibody fragments, such as Fab, $F(ab')_2$, Fv, and sFv fragments. Recombinant, chimeric, and humanized antibodies are also provided.

Recombinant "humanized antibodies" which specifically bind to one of the four plexin polypeptides can be synthesized according to methods known in the art (see, *e.g.*, Green L.L. *et al.*, *Nature Genetics* 7: 13-21, 1994 for fully humanized antibodies expressed in transgenic animals; see also U.S. Patent Nos: 5,693,761; 5,777,085; and 5,585,089). Humanized antibodies are chimeras comprising mostly human IgG sequences into which at least portions of the regions responsible for specific antigen-binding (*e.g.*, CDR's) have been inserted. Animals are immunized with the desired antigen, the corresponding antibodies are isolated, and the portion of the variable region sequences responsible for specific antigen binding are removed. The animal-derived antigen binding regions are then cloned into the appropriate position of human antibody genes in which the antigen binding regions have been deleted. Humanized antibodies minimize the use of heterologous (*i.e.*, inter-species) sequences in human antibodies, and thus are less likely to elicit immune responses in the treated subject (see also, *e.g.*, U.S. Patent No. 5,807,715).

Construction of different classes of recombinant antibodies can also be accomplished by making chimeric or humanized antibodies comprising nonhuman variable domains and human constant domains (CH1, CH2, CH3) isolated from different classes of immunoglobulins. For example, antibodies with increased antigen binding site valencies can be recombinantly produced by cloning the antigen binding site into vectors carrying the human chain constant regions (see, *e.g.*, Arulanandam *et al.*, *J. Exp. Med.* 177: 1439-1450, 1993).

In addition, standard recombinant DNA techniques can be used to alter the binding affinities of recombinant antibodies with their antigens by altering amino acid residues in the vicinity of the antigen binding sites. The antigen binding affinity of a humanized antibody can be increased by mutagenesis based on molecular modeling (see, *e.g.*, Queen et al., *Proc. Natl. Acad. Sci.* 86: 10029-10033, 1989).

Also provided in the invention are plexin polypeptide-specific single polypeptide chain antibodies (see general methods in U.S. Patent Nos. 4,946,788 and

4,704,692); single domain antibodies (Ward, E.S. et al., *Nature* **341**: 544-546, 1989); and chimeric antibodies (U.S. Patent No. 4,816,567).

Binding agents that specifically bind the plexin polypeptides of the present invention are useful, for example, in determining expression levels of the plexin polypeptides in various tissues of the body, Western blotting analysis, and immunochromatography. Particularly, binding agents that specifically bind the plexin polypeptides are useful for binding the plexin polypeptide on a cell expressing the plexin polypeptide, thereby activating the cell.

A binding agent that specifically binds one of the four plexin polypeptides, for example, is effective as an immune modulator. Additional applications include, without limitation, an injectable formulation comprising a binding agent that specifically binds one of the four plexin polypeptides that is useful to antagonize activity in a disease involving aberrant immune regulation or a disease involving aberrant cell growth.

In an eighth aspect, the invention provides an isolated antisense oligonucleotide complementary to a portion of a nucleic acid molecule encoding one of the four plexin polypeptides. In certain embodiments, hybridization of the antisense oligonucleotide to the nucleic acid molecule inhibits transcription or translation of the nucleic acid molecule.

By two nucleic acid molecules being "complementary" to one another is meant that the first nucleic acid molecule (*e.g.*, an oligonucleotide) is able to form Watson-Crick base pair hydrogen bonds (*i.e.*, hybridize) with the second nucleic acid molecule to form a duplex. The first nucleic acid molecule is thus a "complement" of the second nucleic acid molecule.

The antisense oligonucleotides according to the invention are complementary to a region of a nucleic acid molecule (or a region at the intron/exon boundary of DNA or RNA) that encodes one of the four plexin polypeptides. Preparation of antisense oligonucleotides is well known (see, *e.g.*, Agrawal *et al.*, *Trends Biotechnol.* **10**:152-158, 1992; U.S. Patent No. 5,149,798; Agrawal *et al.*, *Proc. Natl. Acad. Sci. USA* **85**:7079-7083, 1988; Froehler, *Tetrahedron Lett.* **27**:5575-5578, 1986; and Bergot et al., *J. Chromatog.* **559**:35-42, 1992.

In a ninth aspect, the invention provides a method for identifying a nucleic acid molecule encoding one of the four plexin polypeptides, comprising contacting a pool of candidate nucleic acid molecules with a nucleic acid molecule encoding one of the four

plexin polypeptides, wherein hybridization of the nucleic acid molecule encoding one of the four plexin polypeptides under stringent conditions to a candidate nucleic acid molecule identifies the candidate nucleic acid molecule as a nucleic acid molecule that encodes one of the four plexin polypeptides. According to this aspect of the invention, "hybridization" and "stringent conditions" are as defined above for the first aspect of the invention. In certain embodiments, the nucleic acid molecule encoding one of the four plexin polypeptides has a nucleic acid sequence comprising SEQ ID NOS: 1, 3, 5 or 7.

It will be understood that the isolated plexin polypeptides according to the second aspect of the invention, the plexin chimeric molecules according to the fifth aspect of the invention, and binding agents that specifically bind the plexin polypeptides according to the seventh aspect of the invention, are useful as therapeutics to treat an individual suffering from, or suspected of having, a disease or disorder involving aberrant immune regulation or an individual suffering from, or suspected of having, a disease or disorder involving aberrant cell growth, particularly nerve cell growth.

By "disease or disorder involving aberrant immune regulation" is meant any disease or disorder in which an abnormal immune response is generated in response to either self or foreign antigens. Thus, this definition includes, without limitation, autoimmune diseases (*e.g.*, lupus, inflammatory bowel disease, or Diabetes Type 1) and immunosuppressive diseases (*e.g.*, multiple sclerosis or rheumatoid arthritis).

By "disease or disorder involving aberrant cell growth" is meant any disease or disorder in which an abnormal amount of cell growth is observed. "Cell growth" is defined above. Thus, diseases and disorders involving aberrant cell growth include hyperplasia, neoplasia, and cancer, as well as degenerative diseases, such as neurodegenerative diseases.

Preferable therapeutically useful plexin polypeptides are soluble polypeptides (*e.g.*, lacking the hydrophobic transmembrane domain of the plexin polypeptides), particularly soluble polypeptide fragments that are secreted by the cell in which the fragment was produced. In a preferred embodiment the soluble plexin polypeptides are selected from the group consisting of plexin-A-1 (Maestrini et al. 1996 *supra*), plexin-A-2 (Maestrini et al. 1996 *supra*), plexin-A-3 (Maestrini et al. 1996 *supra*), plexin-A-4, plexin-B-1 (Maestrini et al. 1996, *supra*), plexin-B-2, plexin-B-3, plexin-C1 (Comeau et al. 1998 *supra*), plexin-D-1.

In a tenth aspect, the invention provides a method for diagnosing a disease involving aberrant immune regulation or a disease involving aberrant cell growth, comprising comparing the amino acid sequence of one of the four plexin polypeptides from an individual suspected of having the disease with the amino acid sequence of one of the four plexin polypeptides from an unaffected individual, wherein the presence of a difference between the two amino acid sequences identifies the individual suspected of having the disease as having the disease. "Disease or disorder involving aberrant immune regulation" and "disease or disorder involving aberrant cell growth" are as defined above.

By "difference" in the amino acid sequence of one of the four plexin polypeptides from an individual suspected of having the disease or disorder as compared with the amino acid sequence of one of the four plexin polypeptides from an unaffected individual, is meant any mutation that changes the amino acid sequence including substitution, deletion, of addition of one or more amino acid residues.

Thus, in one nonlimiting example, one of the four plexin polypeptides is extracted from cells of an individual suspected of having a disease involving aberrant immune regulation (*e.g.*, using an antibody according to the seventh aspect of the invention). The amino acid sequence of the plexin polypeptide is determined by N-terminal sequencing and compared to the amino acid sequence of one of the four plexin polypeptides from an unaffected individual (*i.e.*, a normal individual of the same species that does not have a disease involving aberrant immune regulation or a disease involving aberrant cell growth). If there is a difference in the two amino acid sequences, the individual suspected of having a disease involving aberrant immune regulation is identified as having a disease involving aberrant immune regulation, and may be treated accordingly.

In certain embodiments of the tenth aspect, the amino acid sequence of the plexin polypeptide from the unaffected individual comprises the sequence of SEQ ID NO: 2, 4, 6, 8 or 10.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such

equivalents are considered to be within the scope of this invention, and are covered by the following claims.

Practice of the present invention will employ, unless indicated otherwise, conventional techniques of cell biology, cell culture, molecular biology, microbiology, recombinant DNA, protein chemistry, and immunology, which are within the skill of the art. Such techniques are described in the literature. See, for example, **Molecular Cloning: A Laboratory Manual,** 2nd edition. (Sambrook, Fritsch and Maniatis, eds.), Cold Spring Harbor Laboratory Press, 1989; **DNA Cloning**, Volumes I and II (D.N. Glover, ed), 1985; **Oligonucleotide Synthesis**, (M.J. Gait, ed.), 1984; U.S. Patent No. 4,683,195 (Mullis et al.,); **Nucleic Acid Hybridization** (B.D. Hames and S.J. Higgins, eds.), 1984; Transcription and Translation (B.D. Hames and S.J. Higgins, eds.), 1984; **Culture of Animal Cells** (R.I. Freshney, ed). Alan R. Liss, Inc., 1987; **Immobilized Cells and Enzymes,** IRL Press, 1986; **A Practical Guide to Molecular Cloning** (B. Perbal), 1984; **Methods in Enzymology,** Volumes 154 and 155 (Wu et al., eds), Academic Press, New York; **Gene Transfer Vectors for Mammalian Cells** (J.H. Miller and M.P. Calos, eds.), 1987, Cold Spring Harbor Laboratory; **Immunochemical Methods in Cell and Molecular Biology** (Mayer and Walker, eds.), Academic Press, London, 1987; **Handbook of Experiment Immunology,** Volumes I-IV (D.M. Weir and C.C. Blackwell, eds.), 1986; **Manipulating the Mouse Embryo,** Cold Spring Harbor Laboratory Press, 1986.

The following Examples are provided to illustrate the present invention, and should not be construed as limiting thereof.

## EXAMPLES

Example 1

Identification and cDNA cloning of *plexins* and sequence analysis

Since the coding sequences of human *plexin-B1(SEP), plexin-A2(OCT)* and *plexin-A1(NOV)* were incomplete, we obtained the missing cDNA by RT-PCR; primers were designed by homology to orthologous murine sequences and corresponding ESTs. Updated database entries are X87904, X87831 and X87832, respectively. Partial cDNA of *plexin-A4* was obtained by assembling five overlapping human ESTs (HGI THC Report: THC203425), deriving from chromosome 7 specific cDNA pools. Another EST from chr. 7 (clone 7B19F10) encodes the cytoplasmic domain of a plexin and presumably derives from the same gene as *plexin-A4*. *Plexin-B2* cDNA was amplified by RT-PCR starting from the partial cDNA sequences of clones *MM1* (Shinoura, N., Shamraj, O.I., Hugenholz, H., Zhu, J.G., McBlack, P., Warnick, R., Tew, J.J., Wani, M.A., and Menon, A.G. (1995). Identification and partial sequence of a cDNA that is differentially expressed in human brain tumors. Cancer Lett *89*, 215-221) and KIAA0315 (Genbank database); the genomic locus of *SEP-B* was identified due to its 100% sequence identity with clone C22_311 from human chromosome 22. *Plexin-B3* coding sequence was identified in the genomic sequence of ALD locus on human chromosome Xq28, using the algorithms HEXON and GENIE. *Plexin-D1* was similarly found in the genomic sequence of chromosome 3 (pac pDJ70i11). The genomic sequence of *plexin-B1(SEP),* in the region of the alternative splicing of the extracellular domain, was obtained using the following primers: sense 5'GCAGCACCTGTGCACCCACAAGGC3' and antisense: 5'TGCAGGCTGGACGGGAGGATGAGG3'. The common donor site is CCATCAG/gtgattgt (position 2028 from ATG); the alternative splice acceptor sites are: (i) ccccttcag/AGCCC, leading to the canonical plexin-B1 sequence, and (ii) ctcctctcag/GTGAT, leading to "plexin-B1 truncated" variant. All these new sequences were analyzed using the algoritms BLAST2, NETPHOS (phosphorylation prediction sites, by Nicolaj Blom), PH-PREDICT and Consensus Protein Secondary Structure prediction at IBCP. The phylogenetic tree was generated using AllAll algorithm of the Darwin sequence analysis system (at CBRG).

Example 2

*Plexin* cDNA expression constructs and protein analysis

Cell transfections were carried out by Calcium phosphate and DEAE-dextran methods, using 5-10 μg of each cDNA (1-2 μg each in case of cotransfections). For transient transfections in COS and BOSC-23 cells the cDNA was cloned in pCDNA3 or derived expression plasmids (Invitrogen). MDCK stable transfectants for *plexin-A3* were obtained using pCEP4 expression plasmid (Invitrogen); the selection was done in the presence of Hygromicin-B (100-200 μg/ml). Plexin-A3 positive clones of MDCK cells were isolated from two independent transfections, and showed identical biological properties. Plexin and neuropilin expression constructs included a VSV- and myc-tag at the N' and C' protein termini, respectively, detected by monoclonal antibodies anti-VSV-G (cat. V-5507, Sigma) and anti-cMyc-tag (cat. OP10-100UG, Calbiochem). "Plexin-B1 truncated" splice variant was expressed from a cDNA fragment isolated by RT-PCR and VSV-tagged at the N' terminus: the encoded amino acid sequence spans up to aa 676 (including the *sema* domain and two MRS motifs). "Plexin-B1-sema" derives from a further deletion of the plexin-B1 extracellular domain, and exclusively includes the *sema* domain. "Plexin-B1-Δsema" protein mutant includes only the C' terminal half of plexin-B1 extracellular domain, starting from amino acid 606, i.e. excluding sema domain and first MRS but including second and third MRS, transmembrane and intracellular domains.

For immunoprecipitations, cells were lysed with EB buffer (20 mM Tris-HCl pH 7.4, 5 mM EDTA, 150 mM NaCl, 10% glycerol, 1% Triton X-100), in the presence of a cocktail of protease inhibitors and 1mM Na-ortovanadate. Immunoprecipitations were performed at 4□C for 4h with the appropriate antibodies; high stringency washes were performed, in the presence of 1 M LiCl.

For *in vitro* kinase assays, immunopurified proteins were incubated with kinase buffer (50 mM Hepes, 100 μM DTT, 5 mM $MnCl_2$, 5 mM $MgCl_2$) in the presence of redivue 5 μCi [$\gamma$-$^{32}$P] ATP (Amersham) for 10 minutes at 4°C in agitation. Samples were then submitted to SDS-PAGE and autoradiography, or analysed using a Phosphor-Imager system (Molecular Dynamics). Alkali treatment of the polyacrilamide gels was performed with 1M KOH for two hours at 55°C.

Western blots were performed according to standard methods. Specific detection of phospho-tyrosines was done with PY20 MoAb (Trasduction laboratories). Final detection was done with ECL system (Amersham).

Example 3

Semaphorin-SEAP binding assays

Soluble forms of Semaphorin extracellular domains were expressed as chimeric molecules with placental Secreted Alkaline Phosphatase (SEAP) and harvested from the conditioned media of transiently transfected COS or BOSC-23 cells. Serum-free media were concentrated over 100 times using Centricon Plus-20 filters (Millipore) with a molecular weight cutoff of 100 kDa. The AP activity of these media was assessed as described (Flanagan, J.G. and Leder, P. (1990). The kit ligand: a cell surface molecule altered in steel mutant fibroblasts. Cell *63*, 185-194); the specific activity of chimeric molecules was approx. 1000 U/mg. Concentrated Semaphorin-SEAP were diluted as appropriate in a Hepes buffered saline, additioned with 0.2% BSA, 0.1% $NaN_3$, 5 mM $CaCl_2$ and 1 mM $MgCl_2$ (HBSBA). For binding assays, COS cells transiently transfected with plexins were seeded on 48 well plates to reach confluence, and then incubated with Semaphorin-SEAP preparations (approx 1-5 nM) for 90 minutes at room temperature. The binding was detected as described (Flanagan and Leder, 1990). Binding experiments with plexin-C1/VESPR were as described (Comeau, M.R., Johnson, R., DuBose, R.F., Petersen, M., Gearing, P., VandenBos, T., Park, L., Farrah, T., Buller, R.M., Cohen, J.I., Strockbine, L.D., Rauch, C., and Spriggs, M.K. (1998). A poxvirus-encoded semaphorin induces cytokine production from monocytes and binds to a novel cellular semaphorin receptor, VESPR. Immunity. *8*, 473-482; He, Z. and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. Cell *90*, 739-751).

For *in vitro* binding assays, plexin-B1 was purified from cell extracts by immunoprecipitation with anti-VSV antibody. Extracts of mock-transfected cells were used as control samples. After washing, the immunocomplexes were incubated with serial dilutions of CD100-SEAP (prepared as above) for 2 hours at 4°C, in continuous agitation. Samples were then washed 3 times with HBSBA and the bound alkaline phosphatase activity was measured by colorimetric assay using p-nitro-phenyl-phosphate, as described (Flanagan and Leder, 1990). Scatchard analysis was done using Equilibrate (by GertJan C. Veenstra).

Example 4

In situ hybridization analysis

RNA *in situ* hybridization was performed essentially as described (He, Z. and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. Cell *90*, 739-751). Briefly cDNA fragments of plexin-A1, -A2, and -A3 were used to generate $^{35}$S-labeled antisense and sense RNA probes, which were used for in situ hybridization histochemistry of cryostat sections of rat embryos.

Example 5

Xenopus turning assay

The methods for injecting mRNA encoding various constructs, and for studying the turning responses of the neurons, are exactly as described previously (Hong, K., Hinck, L., Nishiyama, M., Poo, M.M., Tessier-Lavigne, M., and Stein, E. (1999). A ligand-gated association between cytoplasmic domains of UNC5 and DCC family receptors converts netrin-induced growth cone attraction to repulsion. Cell *97*, 927-941); Song, H., Ming, G., He, Z., Lehmann, M., Tessier-Lavigne, M., and Poo, M. (1998). Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides [see comments]. Science *281*, 1515-1518).

Example 6

Mixed-culture assays and time-lapse videomicroscopy

Mock-transfected and plexin-A3 overexpressing MDCK cells were seeded with mesenchymal cells (NIH 3T3, KJ29, D17, among others), in multiwell culture plates by 1:4 or 1:1 ratio. NIH and KJ-29 cells were sometimes labeled by addition of DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, Fluka) in the culture medium, 4 hours before harvesting for the assay; clusters of cells marked with this dye are marked in blue (in light microscopy) and emit red epifluorecence (TRITC filter). The repelling effect was observed 16-30 hours after confluency, by contrast phase microscopy using Leica DM IL. The progress of the assays was also monitored by time-lapse video-microscopy (320 minutes recording were converted into 1 minute play). To determine the time-length of cell contacts, for each assay, randomly chosen fibroblasts were followed during several hours and the duration of each contact between their lamellipodia and MDCK cells was measured. The doubling time of cells and their viability during the assay could also be analyzed, and no differences were observed in presence of control or plexin-A3 expressing cells. Substrate adhesion of plexin-A3 overexpressing MDCKs was analyzed by counting attached cells after 30 minutes from

seeding on micro-wells coated with fibronectin, collagen or polylysin, in the absence of calf serum: no differences versus control cells were observed.

Example 7

Apoptosis detection

TUNEL reaction (Boehringer detection kit) was performed on mixed coltures of MDCK and NIH3T3 cells, 24 hours after seeding in a 24-well culture plate. The labeling was converted into a colorimetric signal for analysis by light microscopy using the TUNEL-AP detection kit (Boehringer). As a positive control for the induction of apoptosis, the same cells were treated with UV-C (50 $\mu J/cm^2$) or 1$\mu$M staurosporin.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be apparent to those skilled in the art that certain changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention, which is delineated by the appended claims.

Example 8

Plexins are specific receptors for cell surface semaphorins in vertebrates

Plexin-C1 (VESPR) has been shown to bind the soluble viral semaphorins Sema VA and VB (Comeau et al., 1998 supra), and we recently found that Drosophila Plexin A (D Plex A) interacts with transmembrane Sema 1a (Winberg et al., 1998 supra). We therefore examined in vertebrates whether the extracellular domain of several different cellular semaphorins -fused to alkaline phosphatase- could bind members of the human plexin-A, -B and -C subfamilies. Multiple secreted semaphorins of class 3 (Sema3A, Sema 3C or Sema3F; see below) did not interact with plexins-A1, -A2, -A3, -B1, B2, or -C1 (data not shown). In contrast, plexin-C1(Vespr) specifically bound Sema7A(Sema-K1) (Fig. 2a), a GPI-membrane linked semaphorin (class 7). This result is not entirely unexpected, since Sema7A may represent the cellular counterpart of viral semaphorin SemaVB, previously shown to interact with this plexin (Comeau et al., 1998 *supra*). More interestingly, the class 4 transmembrane semaphorin Sema4D (CD100) did interact strongly and specifically with plexin-B1 (Fig. 2a). Thus the prototypes of two distinct plexin families are the receptors for members of two distinct semaphorin sub-classes. We also found that Sema7A and Sema4D do not bind to neuropilin-1 or -2 alone, nor did co-transfection of either neuropilin with plexin-B1 significantly modify

its binding efficiency (not shown). Neuropilins thus seem so far to function as receptors only for vertebrate semaphorins of class 3.

The affinity constant of Sema4D for plexin-B1 was estimated by Scatchard plot to be in the subnanomolar range ($K_D$ = 0.9 nM, Fig. 2b; the estimated $K_D$ of Sema7A for plexin-C1 is 2.1 nM, not shown). These values are consistent with those observed for semaphorins-neuropilins, and fly semaphorin1-Plexin A interactions (He and Tessier-Lavigne, 1997 *supra*Winberg et al., 1998).

We used two deletion constructs of plexin-B1 to explore the semaphorin binding sites of plexins. Neither the N-terminal half of plexin-B1 extracellular domain ("plexin-B1-truncated", see previous paragraph), nor its C-terminal half ("plexin-B1-Δsema", see Experimental Procedures) was sufficient alone to bind CD100 (see Fig. 2a), suggesting that the binding of Sema4D depends on multiple structural determinants of the extracellular domain of plexin-B1.

Example 9

Plexins associate with class 3 Semaphorin receptors, Neuropilins

As outlined above, secreted semaphorins of subclass 3 are known to bind neuropilins (He and Tessier-Lavigne, 1997 *supra*; Kolodkin et al., 1997 *supra*; Chen, H., Chedotal, A., He, Z., Goodman, C.S., and Tessier-Lavigne, M. (1997) "Neuropilin-2, a novel member of the neuropilin family, is a high affinity receptor for the semaphorins Sema E and Sema IV but not Sema III." Neuron *19*, 547-559). However, the short cytoplasmic tail of neuropilins seems to be dispensable for their biological activity (Nakamura, et al (1998) *supra)*, indicating the requirement of an associated co-receptor for signal transduction. Interestingly, in *Drosophila* (where neuropilins have not been identified to date) Plexin A is sufficient to mediate the biological response to semaphorin-1 in axon guidance (Winberg et al., 1998*supra*).

In an initial set of experiments, we could not observe binding of the class 3 semaphorins Sema3A(Sema III), Sema3C(Sema E) or Sema3F(Sema IV) to plexins-A1, A2, A3, B1, B2 or C1 (not shown). To test whether plexins might be coreceptors with neuropilins for class 3 semaphorins, we set up co-precipitation experiments in COS cells to test whether neuropilins may interact with plexins. Three tested plexins (plexin-A1, -A3 and -B1) associated both with neuropilin-2 (Np2, shown in Fig. 3) and neuropilin-1 (not shown). The binding was specific, inasmuch as neither neuropilin nor any plexins coimmunoprecipitated with the netrin receptor DCC, under conditions

where DCC coimmunoprecipitated with the other netrin receptor UNC5H2 (Fig. 3 and data not shown). We observed finally that the plexin-neuropilin association is mediated by the *sema domain* of plexins, as demonstrated using either the "plexin-B1 truncated" splice variant (Figure 3) or an even shorter form of the extracellular domain ("plexin-B1-sema", see Experimental procedures, not shown).

To further support the idea of a plexin-neuropilin multimeric receptor complex for semaphorins, we show here that plexin-A3 (e.g.) is expressed in a large number of neuronal classes, including sensory, sympathetic, motor, and olfactory bulb neurons (Figure 4 and data not shown), which are known to respond to class 3 semaphorins, and which express either neuropilin-1 or neuropilin-2 or both (Chen et al., 1997 *supra*; Feiner, L., Koppel, A.M., Kobayashi, H., and Raper, J.A. (1997). Secreted chick semaphorins bind recombinant neuropilin with similar affinities but bind different subsets of neurons in situ. Neuron *19*, 539-545; He and Tessier-Lavigne, 1997 *supra*; Kolodkin et al., 1997 *supra*). Thus, plexin-A3 is a candidate for a physiological coreceptor involved in mediating class 3 semaphorin effects on these axons. Other plexins may also have a role as neuropilin coreceptors in specific cell populations, such as plexin-A2, which is expressed in a subset of sensory neurons and in dorsal horn cells, and plexin-A1, which is expressed at low levels and broadly in the spinal cord (Figure 4).

To directly test the possible involvement of plexins in class 3 semaphorin signal transduction, we studied the repulsive responses of Xenopus spinal neurons to Sema3A, which is mediated by a receptor mechanism involving neuropilin-1 (Song et al., 1998 *supra*). We asked whether these responses could be altered by expression of a presumed dominant-negative plexin-A1 construct lacking the cytoplasmic domain of the protein. Transmembrane proteins can be reliably expressed in these neurons by injecting the encoding mRNA at the developmental two cell stage, allowing the embryos to grow to tadpole stage, and then removing the spinal cord and culturing the neurons (Hong et al., 1999 *supra*). We therefore injected the mRNA encoding the truncated plexin-A1 construct, together with mRNA encoding GFP (as a reporter) and then studied the responses of spinal neurons expressing GFP that were derived from these embryos. Whereas control spinal neurons are repelled by Sema3A (Figure 5A, B and Song et al. 1998 *supra*), neurons from embryos injected with mRNA for truncated plexin-A1 did not respond with either repulsion or attraction to Sema3A (Figure 5C,

D). This blocking effect appeared to be specific, since expression of a different heterologous receptor, UNC5H2, did not impair repulsion by Sema3A (Hong et al., 1997 *supra*), and since expression of the truncated plexin construct did not block attractive responses to netrin-1 (Figure 5E, F). Figure 5G, H quantifies these effects. As can be seen, the effect of Sema3A is completely abolished by the truncated plexin; although there is a slight apparent decrease in the attractive effect of netrin-1 the effect is not statistically significant.

Although we have used a truncated plexin-A1 construct, this construct may be expected to interfere with the function of various plexins, since all the plexins tested (A1, A3 and B1) associated with neuropilin-1. These results support a role for one or more plexins in mediating the repulsive Sema3A signal in the Xenopus spinal neurons.

Example 10

Plexins signal via a novel type of tyrosine phosphorylated cytoplasmic domain

The sequences of plexin cytoplasmic domains are highly conserved among plexins but do not match any known sequences. We found that the plexin-A3 and plexin-B1 proteins are phosphorylated on tyrosine residues when overexpressed in human kidney cells (BOSC-23), as demonstrated using anti-phosphotyrosine antibodies (Fig. 6a). Furthermore, after immunoprecipitation and in vitro kinase assays, plexin-A3 and plexin-B1 became phosphorylated (Fig. 6b). Resistance to an alkali treatment (see Experimental procedures) confirmed the specific phosphorylation of tyrosine residues.

The cytoplasmic domains of several receptors, including Met proteins, become tyrosine phosphorylated owing to an intrinsic kinase activity (Ullrich, A. and Schlessinger, J. (1990) "Signal transduction by receptors with tyrosine kinase activity." Cell *61*, 203-212). Since the cytoplasmic domain of plexins is not similar to any bona fide or atypical tyrosine kinase, this suggests that a distinct tyrosine kinase co-immunoprecipitates in association with plexins, and is responsible for their tyrosine phosphorylation. Although some additional phosphorylated proteins can be found specifically with plexin-A3 and -B1, we have not as yet identified this associated kinase. A number of endogenously expressed tyrosine kinases, namely Met, Ron, Abl and Src, were not found associated with plexin-A3 by immunoprecipitation and Western blotting (not shown). Since tyrosine phosphorylated residues often function as docking sites for intracellular signal transducers (Cantley, L.C., Auger, K.R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R., and Soltoff, S. (1991) "Oncogenes and

signal transduction." Cell *64*, 281-302), the fact that the cytoplasmic domains of plexins are tyrosine phosphorylated further suggests that they are part of signaling complexes.

Example 11

Plexin-A3 expressing cells induce repulsion of co-cultured cells

Stable transfectants expressing recombinant human plexin-A3 were successfully obtained in four different cell lines: IMR32 and AF8 (human neuroblasts), and BOSC-23 and MDCK (human and canine kidney cells, respectively). We observed modest phenotypic changes in the transfected cells, which generally become flatter and larger in size. The growth rate of plexin-A3 overexpressing cells was comparable to parental lines and we did not observe differences in the ability to adhere on different substrates (data not shown).

In keeping with previous report on the related Plexin of *Xenopus laevis* (Ohta, K., Mizutani, A., Kawakami, A., Murakami, Y., Kasuya, Y., Takagi, S., Tanaka, H., and Fujisawa, H. (1995). "Plexin: a novel neuronal cell surface molecule that mediates cell adhesion via a homophilic binding mechanism in the presence of calcium ions." Neuron *14*, 1189-1199), we observed a modest increase in calcium-dependent homotypic cell aggregation of plexin-A3 transfectants (not shown). Surprisingly, we found that epithelial MDCK cells overexpressing plexin-A3 mediate strong repelling cues for adjacent cells. This was observed by co-culturing mock-transfected and plexin-A3 overexpressing MDCK cells together with several non-epithelial cell lines (such as NIH3T3, Kj29, and D17; Fig. 7A). Mock MDCKs grew alongside mesenchymal cells until confluency, when both cell types stopped proliferating. In contrast, when plexin-A3-overexpressing epithelial cells were grown in the same conditions, the adjacent mesenchymal cells withdrew from them, and ultimately detached from the plate.

To analyze the dynamics of this repulsion process, we monitored for 36 hours, by time-lapse video-microscopy, mixed cultures of transfected MDCK cells and fibroblasts, in a number of independent experiments. At low cell density, fibroblasts showed intrinsic motility, exploring the surface of the plate with long lamellipodia and filopodia, and thus coming in contacts with a high number of stationary MDCK islets. The time-length of the contacts between fibroblasts and control MDCK cells varied from 30 minutes to several hours, lasting mostly over 100 minutes. However, when fibroblasts were cultured with MDCK cells overexpressing plexin-A3, transient

contacts were observed, often lasting less than 30 minutes (see Fig. 7C). At higher cell density, fibroblasts stopped and clustered alongside the islands of control MDCKs, whereas they kept moving in a hectic fashion between the islands of plexin-A3 transfected cells (data not shown).

This cell-repelling effect is not due to the release of soluble factors, since exchanging conditioned media between mixed cultures was without effect (not shown). Moreover, the two different cell populations grew normally until they came into contact, indicating that the repelling effect requires cell-cell interaction. To rule out the possibility that plexin-A3 expressing cells generate an apoptotic signal for fibroblasts, we monitored cell viability and apoptosis by TUNEL staining. As shown in Figure 7B, the clusters of repelled fibroblasts did not include apoptotic cells; furthermore, the detaching cells still excluded Trypan blue stain and were able to spread again on a new culture plate (not shown).

Taken together, these results demonstrate that in our experimental system, plexin-A3 mediates cell repelling cues, presumably by interacting with surface bound ligands on opposing cells. We could not identify –so far- the specific ligand for plexin-A3, however we propose that this may be a transmembrane semaphorin. It should be noted that the intracellular domains of transmembrane semaphorins, such as Sema4D, also include tyrosine residues, which may themselves become phosphorylated and associate with cytoplasmic signal transducer molecules, a property shown for ligands of the ephrin family (Holland, S.J., Gale, N.W., Mbamalu, G., Yancopoulos, G.D., Henkemeyer, M., and Pawson, T. (1996) "Bidirectional signalling through the EPH-family receptor Nuk and its transmembrane ligands." Nature *383*, 722-725).

What is claimed is:

1. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of the amino acid sequence shown in (SEQ ID NO: 2 (plexin B-2)), (SEQ ID NO: 4 (plexin B-3)), (SEQ ID NO: 6 (plexin D-1)) and (SEQ ID NO: 8 (plexin A-4))
2. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence selected from the group consisting of the nucleotide sequence shown (SEQ ID NO: 1 (plexin B-2)), (SEQ ID NO: 3 (plexin B-3)), (SEQ ID NO: 5 (plexin D-1)) and (SEQ ID NO: 7 (plexin A-4)).
3. A vector comprising the nucleic acid of any one of claims 1 or 2.
4. An isolated polypeptide having at least 80% amino acid sequence identity to an amino acid sequence selected from the group consisting of the amino acid sequence shown in (SEQ ID NO: 2 (plexin B-2)), (SEQ ID NO: 4 (plexin B-3)), (SEQ ID NO: 6 (plexin D-1)) and (SEQ ID NO: 8 (plexin A-4)).
5. An isolated polypeptide having at least 80% amino acid sequence identity to:

(a) the polypeptide shown in (SEQ ID NO: 2 (plexin B-2)), (SEQ ID NO: 4 (plexin B-3)), (SEQ ID NO: 6 (plexin D-1)) and (SEQ ID NO: 8 (plexin A-4)), lacking its associated signal peptide;

(b) an extracellular domain of the polypeptide shown in (SEQ ID NO: 2 (plexin B-2)), (SEQ ID NO: 4 (plexin B-3)), (SEQ ID NO: 6 (plexin D-1)) and (SEQ ID NO: 8 (plexin A-4)), with its associated signal peptide; or

(c) an extracellular domain of the polypeptide shown in (SEQ ID NO: 2 (plexin B-2)), (SEQ ID NO: 4 (plexin B-3)), (SEQ ID NO: 6 (plexin D-1)) and (SEQ ID NO: 8 (plexin A-4)), lacking its associated signal peptide.

6. A chimeric molecule comprising a polypeptide according to claim 4 or 5 fused to a heterologous amino acid sequence.
7. The chimeric molecule of claim 6, wherein the heterologous amino acid sequence is a Fc region of an immunoglobulin.
8. An antibody that specifically binds to a polypeptide according to claim 4 or 5.
9. The antibody according to claim 8, wherein the antibody is a monoclonal, a humanized antibody or a single-chain antibody.
10. A method of suppressing or altering aberrant cell growth involving a signaling pathway between a plexin and a neuropilin in a mammal comprising the step of administering an effective amount of an agent to said mammal capable of interfering with the association between the plexin and neuropilin
11. A method of treating, suppressing or altering a disorder involving aberrant immune regulation involving a signaling pathway between a plexin and a neuropilin in a mammal comprising the step of administering an effective amount of an agent to said mammal capable of interfering with the association between the plexin and neuropilin.
12. The method according to claim 10 or 11 wherein said agent is a chimeric molecule according to claim 6 or 7.
13. The method according to claim 10 or 11, wherein said agent is an antibody according to claim 8 or 9.
14. A method of diagnosing or screening for tumors in a subject characterized by the expression profiles of the polypeptides according to claim 4 or 5 wherein the expression profile of the polypeptides is different in a non-tumor sample as compared to the expression profile of the polypeptides in a tumor sample.

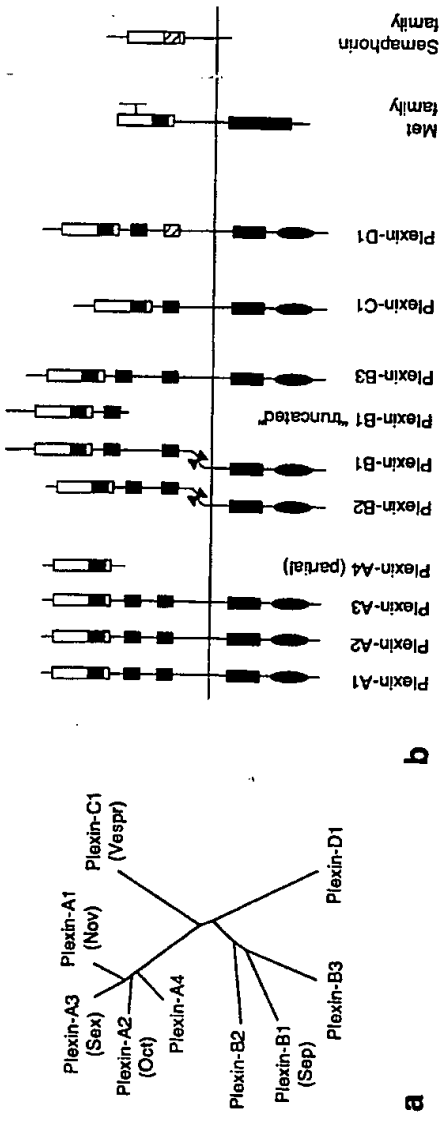
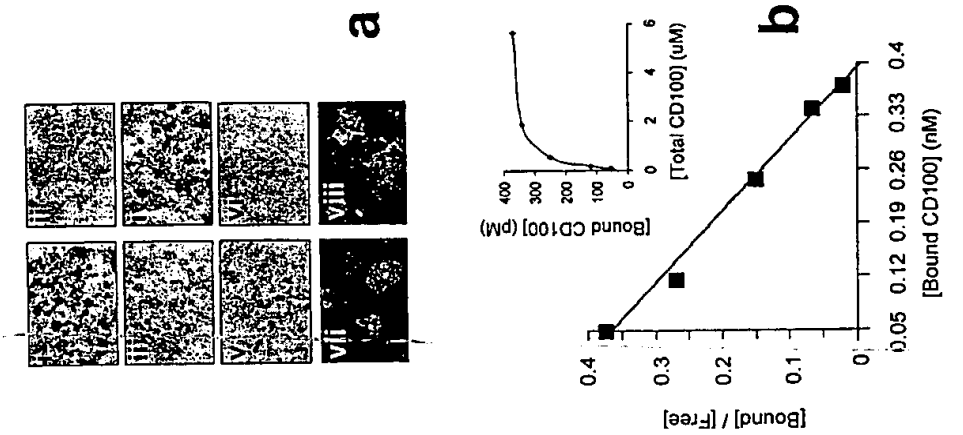



Figure 1 (Tamagnone et al.)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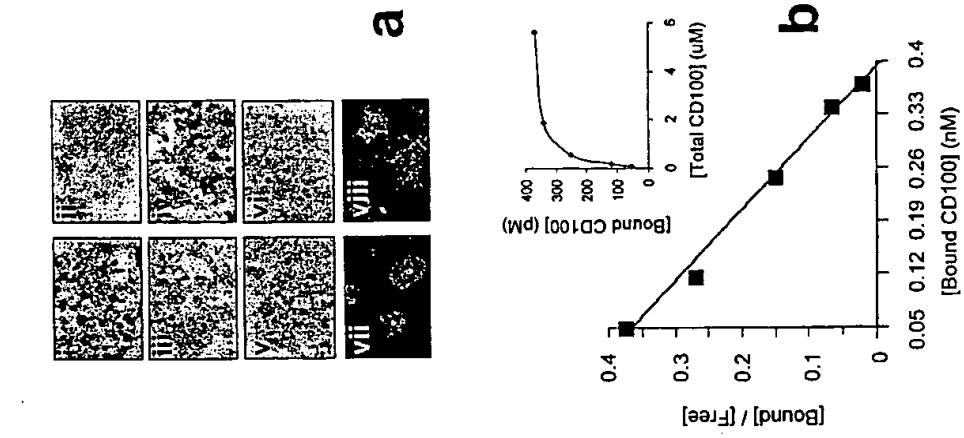
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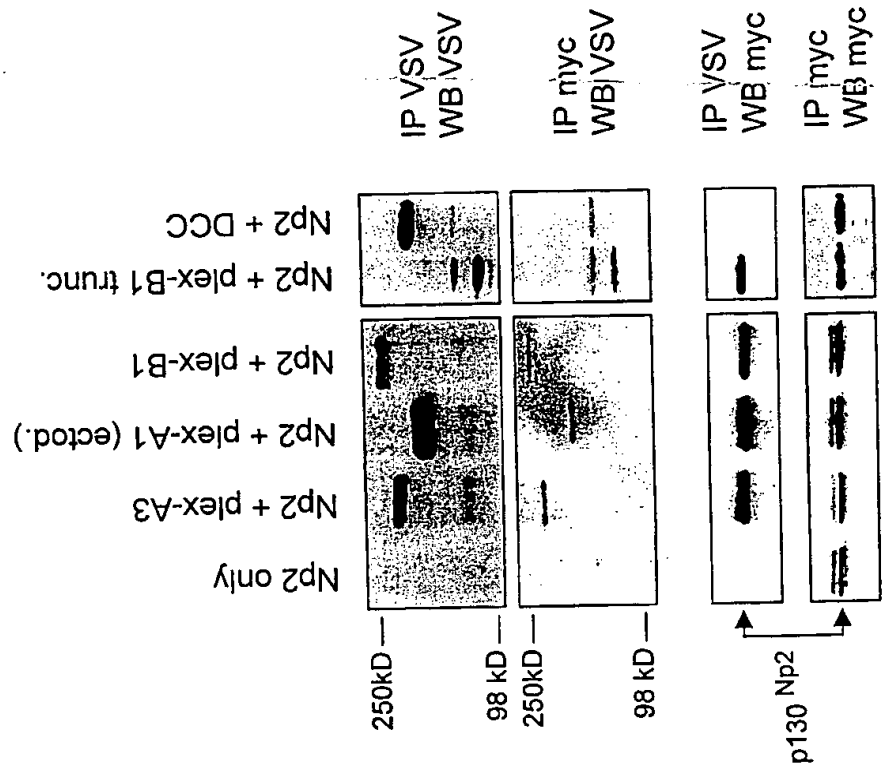
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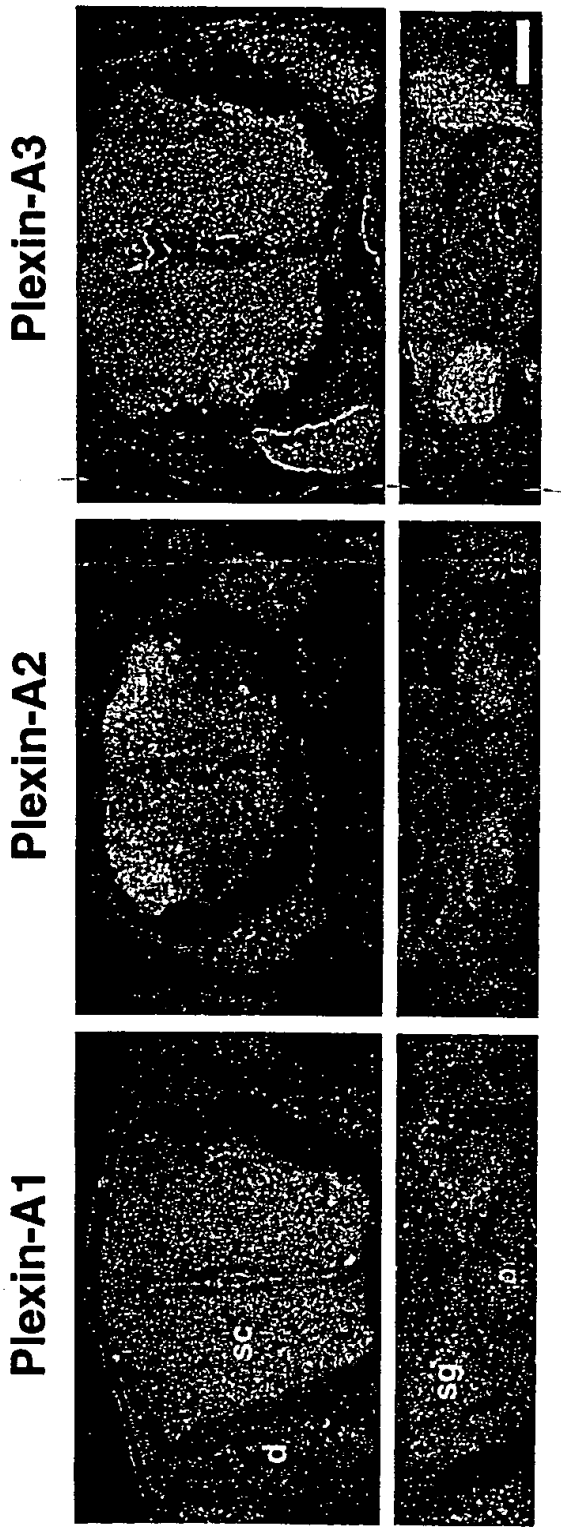
Fig. 3

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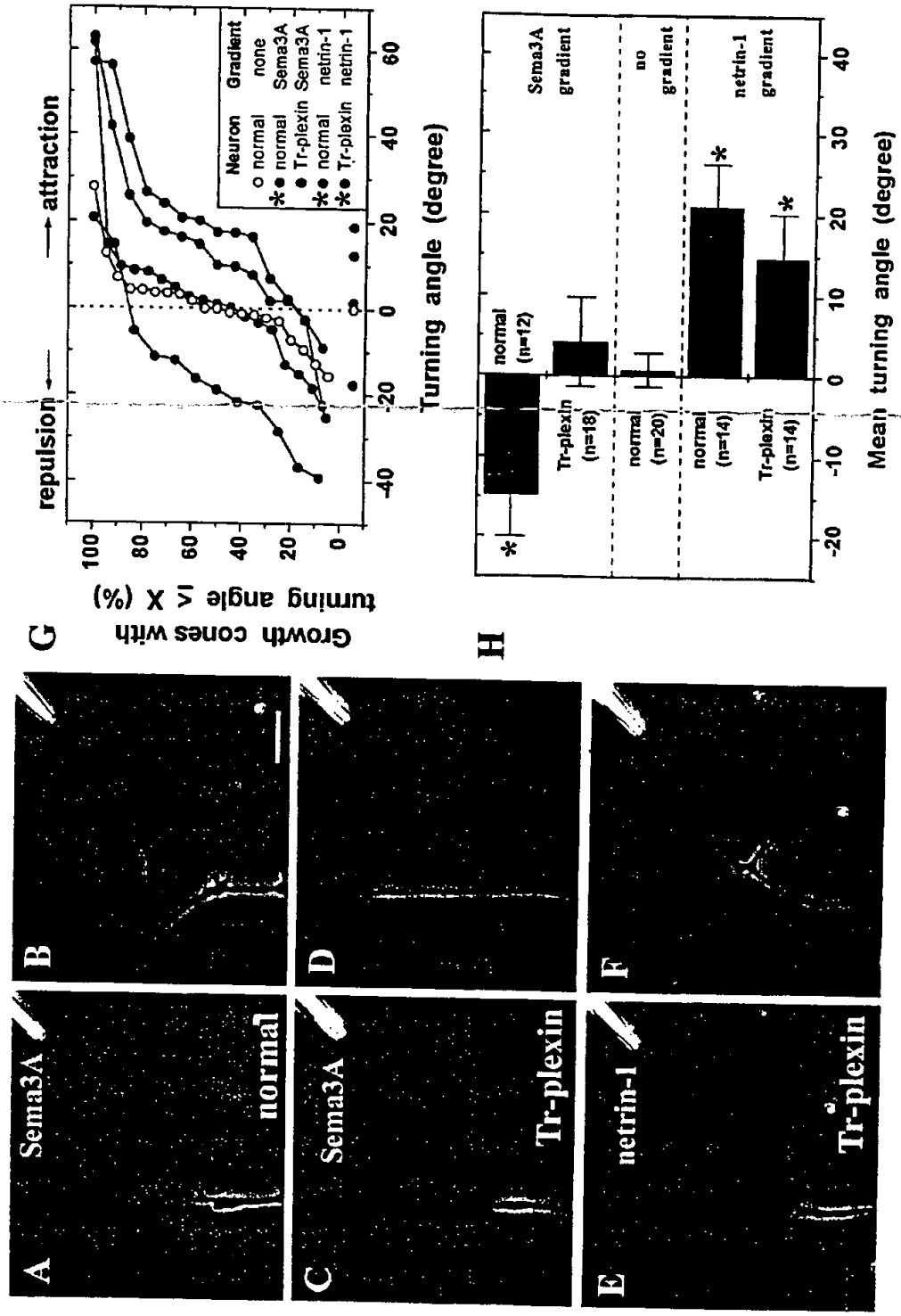
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Turning angle (degree)
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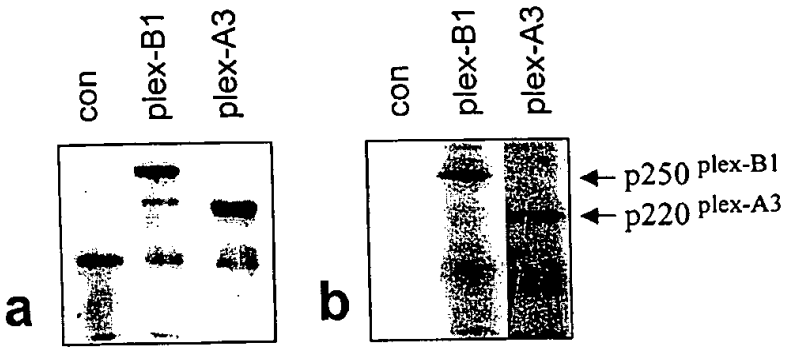
FIG. 5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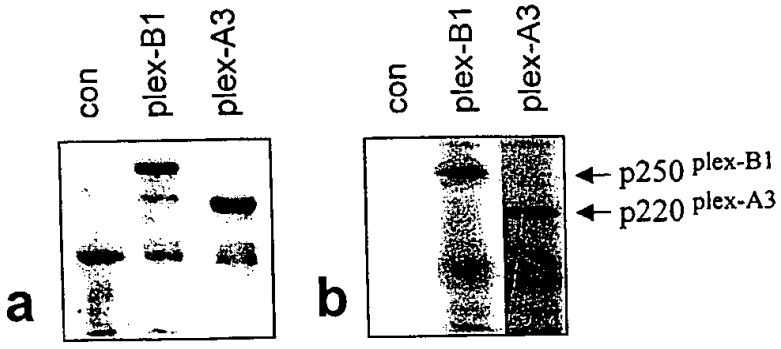
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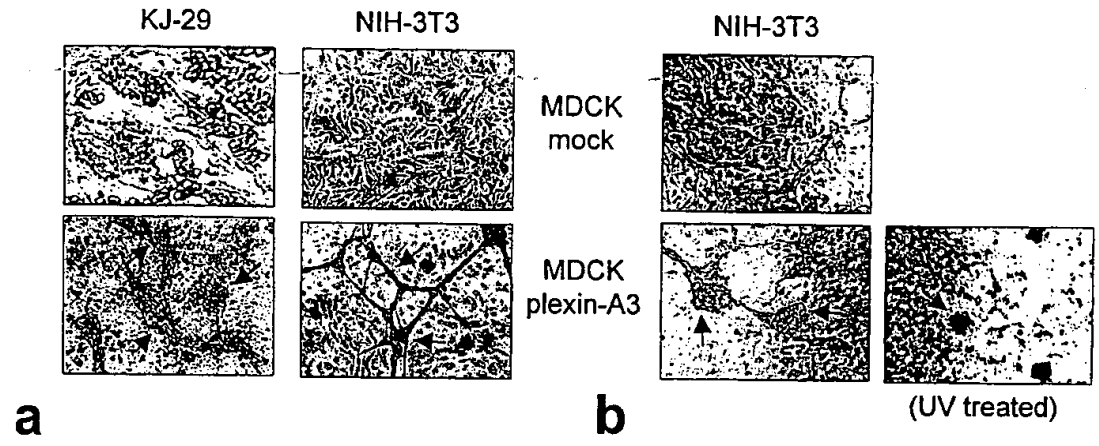
Fig. 6

7/7

FIG. 7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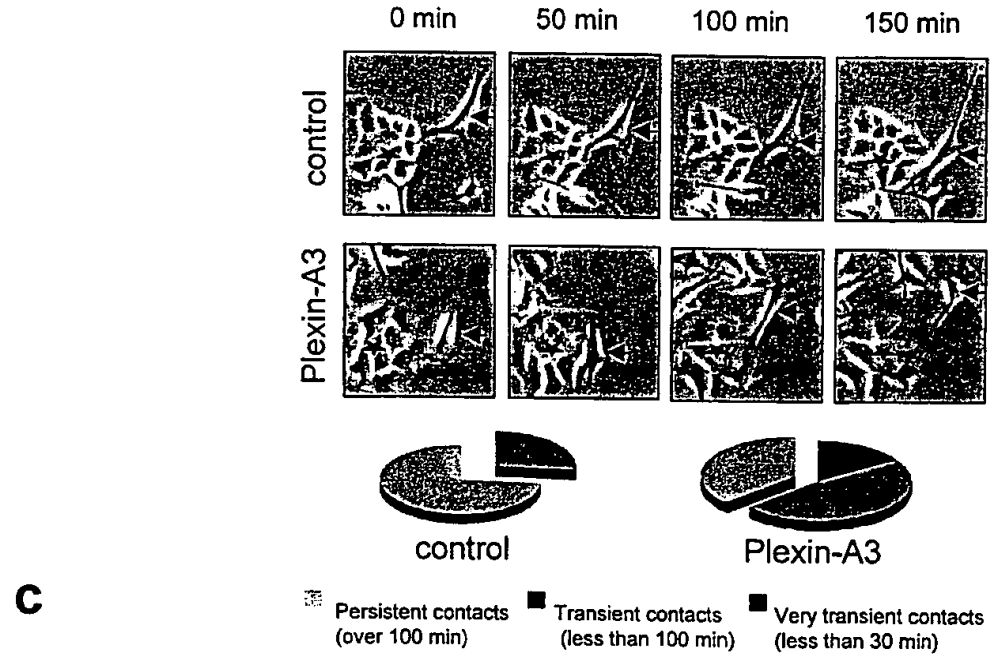
a
b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100 min
150 min
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control
Plexin-A3
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c

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```
        195                 200                 205
Gly Tyr Leu Ser Thr Asn Thr Gln Gln Phe Val Ala Ala Phe Glu Asp
    210                 215                 220
Gly Pro Tyr Val Phe Phe Val Phe Asn Gln Gln Asp Lys His Pro Ala
225                 230                 235                 240
Arg Asn Arg Thr Leu Leu Ala Arg Met Cys Arg Glu Asp Pro Asn Tyr
                245                 250                 255
Tyr Ser Tyr Leu Glu Met Asp Leu Gln Cys Arg Asp Pro Asp Ile His
            260                 265                 270
Ala Ala Ala Phe Gly Thr Cys Leu Ala Ala Ser Val Ala Ala Pro Gly
        275                 280                 285
Ser Gly Arg Val Leu Tyr Ala Val Phe Ser Arg Asp Ser Arg Ser Ser
    290                 295                 300
Gly Gly Pro Gly Ala Gly Leu Cys Leu Phe Pro Leu Asp Lys Val His
305                 310                 315                 320
Ala Lys Met Glu Ala Asn Arg Asn Ala Cys Tyr Thr Gly Thr Arg Glu
                325                 330                 335
Ala Arg Asp Ile Phe Tyr Lys Pro Phe His Gly Asp Ile Gln Cys Gly
            340                 345                 350
Gly His Ala Pro Gly Ser Ser Lys Ser Phe Pro Cys Gly Ser Glu His
        355                 360                 365
Leu Pro Tyr Pro Leu Gly Ser Arg Asp Gly Leu Arg Gly Thr Ala Val
    370                 375                 380
Leu Gln Arg Gly Gly Leu Asn Leu Thr Ala Val Thr Val Ala Ala Glu
385                 390                 395                 400
Asn Asn His Thr Val Ala Phe Leu Gly Thr Ser Asp Gly Arg Ile Leu
                405                 410                 415
Lys Val Tyr Leu Thr Pro Asp Gly Thr Ser Ser Glu Tyr Asp Ser Ile
            420                 425                 430
Leu Val Glu Ile Asn Lys Arg Val Lys Arg Asp Leu Val Leu Ser Gly
        435                 440                 445
Asp Leu Gly Ser Leu Tyr Ala Met Thr Gln Asp Lys Val Phe Arg Leu
    450                 455                 460
Pro Val Gln Glu Cys Leu Ser Tyr Pro Thr Cys Thr Gln Cys Arg Asp
465                 470                 475                 480
Ser Gln Asp Pro Tyr Cys Gly Trp Cys Val Val Glu Gly Arg Cys Thr
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Arg Lys Ala Glu Cys Pro Arg Ala Glu Glu Ala Ser His Trp Leu Trp
            500                 505                 510
Ser Arg Ser Lys Ser Cys Val Ala Val Thr Ser Ala Gln Pro Gln Asn
        515                 520                 525
Met Ser Arg Arg Ala Gln Gly Glu Val Gln Leu Thr Val Ser Pro Leu
    530                 535                 540
Pro Ala Leu Ser Glu Glu Asp Glu Leu Leu Cys Leu Phe Gly Glu Ser
545                 550                 555                 560
Pro Pro His Pro Ala Arg Val Glu Gly Glu Ala Val Ile Cys Asn Ser
                565                 570                 575
Pro Ser Ser Ile Pro Val Thr Pro Pro Gly Gln Asp His Val Ala Val
            580                 585                 590
Thr Ile Gln Leu Leu Leu Arg Arg Gly Asn Ile Phe Leu Thr Ser Tyr
        595                 600                 605
Gln Tyr Pro Phe Tyr Asp Cys Arg Gln Ala Met Ser Leu Glu Glu Asn
    610                 615                 620
Leu Pro Cys Ile Ser Cys Val Ser Asn Arg Trp Thr Cys Gln Trp Asp
625                 630                 635                 640
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Leu Arg Tyr His Glu Cys Arg Glu Ala Ser Pro Asn Pro Glu Asp Gly
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Ile Val Arg Ala His Met Glu Asp Ser Cys Pro Gln Phe Leu Gly Pro
660 665 670
Ser Pro Leu Val Ile Pro Met Asn His Glu Thr Asp Val Asn Phe Gln
675 680 685
Gly Lys Asn Leu Asp Thr Val Lys Gly Ser Ser Leu His Val Gly Ser
690 695 700
Asp Leu Leu Lys Phe Met Glu Pro Val Thr Met Gln Glu Ser Gly Thr
705 710 715 720
Phe Ala Phe Arg Thr Pro Lys Leu Ser His Asp Ala Asn Glu Thr Leu
725 730 735
Pro Leu His Leu Tyr Val Lys Ser Tyr Gly Lys Asn Ile Asp Ser Lys
740 745 750
Leu His Val Thr Leu Tyr Asn Cys Ser Phe Gly Arg Ser Asp Cys Ser
755 760 765
Leu Cys Arg Ala Ala Asn Pro Asp Tyr Arg Cys Ala Trp Cys Gly Gly
770 775 780
Gln Ser Arg Cys Val Tyr Glu Ala Leu Cys Asn Thr Thr Ser Glu Cys
785 790 795 800
Pro Pro Pro Val Ile Thr Arg Ile Gln Pro Glu Thr Gly Pro Leu Gly
805 810 815
Gly Gly Ile Arg Ile Thr Ile Leu Gly Ser Asn Leu Gly Val Gln Ala
820 825 830
Gly Asp Ile Gln Arg Ile Ser Val Ala Gly Arg Asn Cys Ser Phe Gln
835 840 845
Pro Glu Arg Tyr Ser Val Ser Thr Arg Ile Val Cys Val Ile Glu Ala
850 855 860
Ala Glu Thr Pro Phe Thr Gly Gly Val Glu Val Asp Val Phe Gly Lys
865 870 875 880
Leu Gly Arg Ser Pro Pro Asn Val Gln Phe Thr Phe Gln Gln Pro Lys
885 890 895
Pro Leu Ser Val Glu Pro Gln Gln Gly Pro Gln Ala Gly Gly Thr Thr
900 905 910
Leu Thr Ile His Gly Thr His Leu Asp Thr Gly Ser Gln Glu Asp Val
915 920 925
Arg Val Thr Leu Asn Gly Val Pro Cys Lys Val Thr Lys Phe Gly Ala
930 935 940
Gln Leu Gln Cys Val Thr Gly Pro Gln Ala Thr Arg Gly Gln Met Leu
945 950 955 960
Leu Glu Val Ser Tyr Gly Gly Ser Pro Val Pro Asn Pro Gly Ile Phe
965 970 975
Phe Thr Tyr Arg Glu Asn Pro Val Leu Arg Ala Phe Glu Pro Leu Arg
980 985 990
Ser Phe Ala Ser Gly Gly Arg Ser Ile Asn Val Thr Gly Gln Gly Phe
995 1000 1005
Ser Leu Ile Gln Arg Phe Ala Met Val Val Ile Ala Glu Pro Leu Gln
1010 1015 1020
Ser Trp Gln Pro Pro Arg Glu Ala Glu Ser Leu Gln Pro Met Thr Val
1025 1030 1035 1040
Val Gly Thr Asp Tyr Val Phe His Asn Asp Thr Lys Val Val Phe Leu
1045 1050 1055
Ser Pro Ala Val Pro Glu Glu Pro Glu Ala Tyr Asn Leu Thr Val Leu
1060 1065 1070
Ile Glu Met Asp Gly His Arg Ala Leu Leu Arg Thr Glu Ala Gly Ala

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        1075                1080                1085
Phe Glu Tyr Val Pro Asp Pro Thr Phe Glu Asn Phe Thr Gly Gly Val
    1090                1095                1100
Lys Lys Gln Val Asn Lys Leu Ile His Ala Arg Gly Thr Asn Leu Asn
1105                1110                1115                1120
Lys Ala Met Thr Leu Gln Glu Ala Glu Ala Phe Val Gly Ala Glu Arg
                1125                1130                1135
Cys Thr Met Lys Thr Leu Thr Glu Thr Asp Leu Tyr Cys Glu Pro Pro
            1140                1145                1150
Glu Val Gln Pro Pro Pro Lys Arg Arg Gln Lys Arg Asp Thr Thr His
        1155                1160                1165
Asn Leu Pro Glu Phe Ile Val Lys Phe Gly Ser Arg Glu Trp Val Leu
    1170                1175                1180
Gly Arg Val Glu Tyr Asp Thr Arg Val Ser Asp Val Pro Leu Ser Leu
1185                1190                1195                1200
Ile Leu Pro Leu Val Ile Val Pro Met Val Val Val Ile Ala Val Ser
                1205                1210                1215
Val Tyr Cys Tyr Trp Arg Lys Ser Gln Gln Ala Glu Arg Glu Tyr Glu
            1220                1225                1230
Lys Ile Lys Ser Gln Leu Glu Gly Leu Glu Glu Ser Val Arg Asp Arg
        1235                1240                1245
Cys Lys Lys Glu Phe Thr Asp Leu Met Ile Glu Met Glu Asp Gln Thr
    1250                1255                1260
Asn Asp Val His Glu Ala Gly Ile Pro Val Leu Asp Tyr Lys Thr Tyr
1265                1270                1275                1280
Thr Asp Arg Val Phe Phe Leu Pro Ser Lys Asp Gly Asp Lys Asp Val
                1285                1290                1295
Met Ile Thr Gly Lys Leu Asp Ile Pro Glu Pro Arg Arg Pro Val Val
            1300                1305                1310
Glu Gln Ala Leu Tyr Gln Phe Ser Asn Leu Leu Asn Ser Lys Ser Phe
        1315                1320                1325
Leu Ile Asn Phe Ile His Thr Leu Glu Asn Gln Arg Glu Phe Ser Ala
    1330                1335                1340
Arg Ala Lys Val Tyr Phe Ala Ser Leu Leu Thr Val Ala Leu His Gly
1345                1350                1355                1360
Lys Leu Glu Tyr Tyr Thr Asp Ile Met His Thr Leu Phe Leu Glu Leu
                1365                1370                1375
Leu Glu Gln Tyr Val Val Ala Lys Asn Pro Lys Leu Met Leu Arg Arg
            1380                1385                1390
Ser Glu Thr Val Val Glu Arg Met Leu Ser Asn Trp Met Ser Ile Cys
        1395                1400                1405
Leu Tyr Gln Tyr Leu Lys Asp Ser Ala Gly Glu Pro Leu Tyr Lys Leu
    1410                1415                1420
Phe Lys Ala Ile Lys His Gln Val Glu Lys Gly Pro Val Asp Ala Val
1425                1430                1435                1440
Gln Lys Lys Ala Lys Tyr Thr Leu Asn Asp Thr Gly Leu Leu Gly Asp
                1445                1450                1455
Asp Val Glu Tyr Ala Pro Leu Thr Val Ser Val Ile Val Gln Asp Glu
            1460                1465                1470
Gly Val Asp Ala Ile Pro Val Lys Val Leu Asn Cys Asp Thr Ile Ser
        1475                1480                1485
Gln Val Lys Glu Lys Ile Ile Asp Gln Val Tyr Arg Gly Gln Pro Cys
    1490                1495                1500
Ser Cys Trp Pro Arg Pro Asp Ser Val Val Leu Glu Trp Arg Pro Gly
1505                1510                1515                1520
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Ser Thr Ala Gln Ile Leu Ser Asp Leu Asp Leu Thr Ser Gln Arg Glu
                1525                1530                1535
Gly Arg Trp Lys Arg Val Asn Thr Leu Met His Tyr Asn Val Arg Asp
            1540                1545                1550
Gly Ala Thr Leu Ile Leu Ser Lys Val Gly Val Ser Gln Gln Pro Glu
        1555                1560                1565
Asp Ser Gln Gln Asp Leu Pro Gly Glu Arg His Ala Leu Leu Glu Glu
    1570                1575                1580
Glu Asn Arg Val Trp His Leu Val Arg Pro Thr Asp Glu Val Asp Glu
1585                1590                1595                1600
Gly Lys Ser Lys Arg Gly Ser Val Lys Glu Lys Glu Arg Thr Lys Ala
                1605                1610                1615
Ile Thr Glu Ile Tyr Leu Thr Arg Leu Leu Ser Val Lys Gly Thr Leu
            1620                1625                1630
Gln Gln Phe Val Asp Asn Phe Phe Gln Ser Val Leu Ala Pro Gly His
        1635                1640                1645
Ala Val Pro Pro Ala Val Lys Tyr Phe Phe Asp Phe Leu Asp Glu Gln
    1650                1655                1660
Ala Glu Lys His Asn Ile Gln Asp Glu Asp Thr Ile His Ile Trp Lys
1665                1670                1675                1680
Thr Asn Ser Leu Pro Leu Arg Phe Trp Val Asn Ile Leu Lys Asn Pro
                1685                1690                1695
His Phe Ile Phe Asp Val His Val His Glu Val Val Asp Ala Ser Leu
            1700                1705                1710
Ser Val Ile Ala Gln Thr Phe Met Asp Ala Cys Thr Arg Thr Glu His
        1715                1720                1725
Lys Leu Ser Arg Asp Ser Pro Ser Asn Lys Leu Leu Tyr Ala Lys Glu
    1730                1735                1740
Ile Ser Thr Tyr Lys Lys Met Val Glu Asp Tyr Tyr Lys Gly Ile Arg
1745                1750                1755                1760
Gln Met Val Gln Val Ser Asp Gln Asp Met Asn Thr His Leu Ala Glu
                1765                1770                1775
Ile Ser Arg Ala His Thr Asp Ser Leu Asn Thr Leu Val Ala Leu His
            1780                1785                1790
Gln Leu Tyr Gln Tyr Thr Gln Lys Tyr Tyr Asp Glu Ile Ile Asn Ala
        1795                1800                1805
Leu Glu Glu Asp Pro Ala Ala Gln Lys Met Gln Leu Ala Phe Arg Leu
    1810                1815                1820
Gln Gln Ile Ala Ala Ala Leu Glu Asn Lys Val Thr Asp Leu
1825                1830                1835
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<210> 3
<211> 5367
<212> DNA
<213> HOMO SAPIEN

<400> 3

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ctggcacctg gccgaggcac actctatgtc ggcgcagtga accgcctctt ccagctcagc     180
cccgagctgc agctcgaggc cgtggctgtc actggccctg taatcgacag ccctgactgc     240
gtgcccttcc gtgacccagc cgagtgccca caggcccagc tcactgacaa tgccaaccag     300
ctgctgctgg tgagcagccg cgcccaggag ctggtggcct gcgggcaggt gcggcagggc     360
gtgtgtgaga cacggcgcct tggggatgtg gccgaggtgc tgtaccaggc tgaggaccct     420
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```

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<210> 4
<211> 1788
<212> PRT
<213> HOMO SAPIEN

<400> 4

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            20                  25                  30
Asn Thr Thr Leu Asn His Leu Ala Leu Ala Pro Gly Arg Gly Thr Leu
        35                  40                  45
Tyr Val Gly Ala Val Asn Arg Leu Phe Gln Leu Ser Pro Glu Leu Gln
    50                  55                  60
Leu Glu Ala Val Ala Val Thr Gly Pro Val Ile Asp Ser Pro Asp Cys
65                  70                  75                  80
Val Pro Phe Arg Asp Pro Ala Glu Cys Pro Gln Ala Gln Leu Thr Asp
                85                  90                  95
Asn Ala Asn Gln Leu Leu Leu Val Ser Ser Arg Ala Gln Glu Leu Val
            100                 105                 110
Ala Cys Gly Gln Val Arg Gln Gly Val Cys Glu Thr Arg Arg Leu Gly
        115                 120                 125
Asp Val Ala Glu Val Leu Tyr Gln Ala Glu Asp Pro Gly Asp Gly Gln
    130                 135                 140
Phe Val Ala Ala Asn Thr Pro Gly Val Ala Thr Val Gly Leu Val Val
```

```
145                 150                 155                 160
Pro Leu Pro Gly Arg Asp Leu Leu Leu Val Ala Arg Gly Leu Ala Gly
                165                 170                 175
Lys Leu Ser Ala Gly Val Pro Pro Leu Ala Ile Arg Gln Leu Ala Gly
            180                 185                 190
Ser Gln Pro Phe Ser Ser Glu Gly Leu Gly Arg Leu Val Val Gly Asp
        195                 200                 205
Phe Ser Asp Tyr Asn Asn Ser Tyr Val Gly Ala Phe Ala Asp Ala Arg
    210                 215                 220
Ser Ala Tyr Phe Val Phe Arg Arg Arg Gly Ala Arg Ala Gln Ala Glu
225                 230                 235                 240
Tyr Arg Ser Tyr Val Ala Arg Val Cys Leu Gly Asp Thr Asn Leu Tyr
                245                 250                 255
Ser Tyr Val Glu Val Pro Leu Ala Cys Gln Gly Gln Gly Leu Ile Gln
            260                 265                 270
Ala Ala Phe Leu Ala Pro Gly Thr Leu Leu Gly Val Phe Ala Ala Gly
        275                 280                 285
Pro Arg Gly Thr Gln Ala Ala Leu Cys Ala Phe Pro Met Val Glu Leu
    290                 295                 300
Gly Ala Ser Met Glu Gln Ala Arg Arg Leu Cys Tyr Thr Ala Gly Gly
305                 310                 315                 320
Arg Gly Pro Ser Gly Ala Glu Glu Ala Thr Val Glu Tyr Gly Val Thr
                325                 330                 335
Ser Arg Cys Val Thr Leu Pro Leu Asp Ser Pro Glu Ser Tyr Pro Cys
            340                 345                 350
Gly Asp Glu His Thr Pro Ser Pro Ile Ala Gly Arg Gln Pro Leu Glu
        355                 360                 365
Val Gln Pro Leu Leu Lys Leu Gly Gln Pro Val Ser Ala Val Ala Ala
    370                 375                 380
Leu Gln Ala Asp Gly His Met Ile Ala Phe Leu Gly Asp Thr Gln Gly
385                 390                 395                 400
Gln Leu Tyr Lys Val Phe Leu His Gly Ser Gln Gly Gln Val Tyr His
                405                 410                 415
Ser Gln Gln Val Gly Pro Pro Gly Ser Ala Ile Ser Pro Asp Leu Leu
            420                 425                 430
Leu Asp Ser Ser Gly Ser His Leu Tyr Val Leu Thr Ala His Gln Val
        435                 440                 445
Asp Arg Ile Pro Val Ala Ala Cys Pro Gln Phe Pro Asp Cys Ala Ser
    450                 455                 460
Cys Leu Gln Ala Gln Asp Pro Leu Cys Gly Trp Cys Val Leu Gln Gly
465                 470                 475                 480
Arg Cys Thr Arg Lys Gly Gln Cys Gly Arg Ala Gly Gln Leu Asn Gln
                485                 490                 495
Trp Leu Trp Ser Tyr Glu Glu Asp Ser His Cys Leu His Ile Gln Ser
            500                 505                 510
Leu Leu Pro Gly His His Pro Arg Gln Glu Gln Gly Gln Val Thr Leu
        515                 520                 525
Ser Val Pro Arg Leu Pro Ile Leu Asp Ala Asp Glu Tyr Phe His Cys
    530                 535                 540
Ala Phe Gly Asp Tyr Asp Ser Leu Ala His Val Glu Gly Pro His Val
545                 550                 555                 560
Ala Cys Val Thr Pro Pro Gln Asp Gln Val Pro Leu Asn Pro Pro Gly
                565                 570                 575
Thr Asp His Val Thr Val Pro Leu Ala Leu Met Phe Glu Asp Val Thr
            580                 585                 590
```

```
Val Ala Ala Thr Asn Phe Ser Phe Tyr Asp Cys Ser Ala Val Gln Ala
        595                 600                 605
Leu Glu Ala Ala Ala Pro Val Leu Pro Gln Gly Leu Pro Ala Ser Phe
    610                 615                 620
His Cys Trp Leu Glu Leu Pro Gly Glu Leu Arg Gly Leu Pro Ala Thr
625                 630                 635                 640
Leu Glu Glu Thr Ala Gly Asp Ser Gly Leu Ile His Cys Gln Ala His
                645                 650                 655
Gln Arg Glu Leu Pro Val Pro Ile Tyr Val Thr Gln Gly Glu Ala Gln
            660                 665                 670
Arg Leu Asp Asn Thr His Ala Leu Tyr Gly Glu Pro Glu Gly Ser Gln
        675                 680                 685
Ala Gly Gly Ala Gly Trp Val Ala Asp Arg Arg Arg Ser Ala His Cys
    690                 695                 700
Leu Thr Leu Pro Ser Asp Pro Val Arg Leu Arg His Gly Pro Pro Gly
705                 710                 715                 720
Leu Gln Pro Leu Pro Ser Gly Gln Gln Glu Pro Gly Leu Pro Val Thr
                725                 730                 735
Ser Pro Ala Pro Gly Pro Gln Thr Pro Ala Ala Arg Pro Gly Trp Ala
            740                 745                 750
Gly Trp Leu Ala Gly His Pro Ala Leu Gln Ser Gly Ala Trp Val Arg
        755                 760                 765
Gly Thr Pro Ser Ala Ile Ile Cys Leu Leu Gln Val Glu Pro Leu Thr
    770                 775                 780
Gly Pro Pro Glu Gly Gly Leu Ala Leu Thr Ile Leu Gly Ser Asn Leu
785                 790                 795                 800
Gly Arg Ala Phe Ala Asp Val Gln Tyr Ala Asp Pro Val Leu Leu Ser
                805                 810                 815
Leu Ser Pro Arg Trp Gly Pro Gln Ala Gly Gly Thr Gln Leu Thr Ile
            820                 825                 830
Arg Gly Gln His Leu Gln Thr Gly Gly Asn Thr Ser Ala Phe Val Gly
        835                 840                 845
Gly Gln Pro Cys Pro Met Gly Gly Arg Leu Ile Arg Val Arg Gly Thr
    850                 855                 860
Gly Leu Asp Val Val Gln Arg Pro Leu Leu Ser Val Trp Leu Glu Ala
865                 870                 875                 880
Asp Ala Glu Val Gln Ala Ser Arg Ala Gln Pro Gln Asp Pro Gln Pro
                885                 890                 895
Arg Arg Ser Cys Gly Ala Pro Ala Ala Asp Pro Gln Ala Cys Ile Gln
            900                 905                 910
Leu Gly Gly Gly Leu Leu Gln Arg Thr Ala Glu Pro Ser Ser Leu His
        915                 920                 925
Leu Trp Ser Ala Leu Asn Ala Pro Gln Cys Ser Thr Val Cys Ser Val
    930                 935                 940
Asn Ser Ser Ser Leu Leu Leu Cys Arg Ser Pro Ala Val Pro Asp Arg
945                 950                 955                 960
Ala His Pro Gln Arg Val Phe Phe Thr Leu Asp Asn Val Gln Val Asp
                965                 970                 975
Phe Ala Ser Ala Ser Gly Gly Gln Gly Phe Leu Tyr Gln Pro Asn Pro
            980                 985                 990
Arg Leu Ala Pro Leu Ser Arg Glu Gly Pro Ala Arg Pro Tyr Arg Leu
        995                 1000                1005
Lys Pro Gly His Val Leu Asp Val Glu Gly Glu Gly Leu Asn Leu Gly
    1010                1015                1020
Ile Ser Lys Glu Glu Val Arg Val His Ile Gly Arg Gly Glu Cys Leu
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Val Lys Thr Leu Thr Arg Thr His Leu Tyr Cys Glu Pro Pro Ala His
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Ala Pro Gln Pro Ala Asn Gly Ser Gly Leu Pro Gln Phe Val Val Gln
            1060                1065                1070
Met Gly Asn Val Gln Leu Ala Leu Gly Pro Val Gln Tyr Glu Ala Glu
        1075                1080                1085
Pro Pro Leu Ser Ala Phe Pro Val Glu Ala Gln Ala Gly Val Gly Met
    1090                1095                1100
Gly Ala Ala Val Leu Ile Ala Ala Val Leu Leu Leu Thr Leu Met Tyr
1105                1110                1115                1120
Arg His Lys Ser Lys Gln Ala Leu Arg Asp Tyr Gln Lys Val Leu Val
                1125                1130                1135
Gln Leu Glu Ser Leu Glu Thr Gly Val Gly Asp Gln Cys Arg Lys Glu
            1140                1145                1150
Phe Thr Asp Leu Met Thr Glu Met Thr Asp Leu Ser Ser Asp Leu Glu
        1155                1160                1165
Gly Ser Gly Ile Pro Phe Leu Asp Tyr Arg Thr Tyr Ala Glu Arg Ala
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Phe Phe Pro Gly His Gly Gly Cys Pro Leu Gln Pro Lys Pro Glu Gly
1185                1190                1195                1200
Pro Gly Glu Asp Gly His Cys Ala Thr Val Arg Gln Gly Leu Thr Gln
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Val Trp Arg Glu Cys Pro Val Gly Lys Glu Val Gly Leu Gly Asn Tyr
        1235                1240                1245
Trp Pro Glu Thr Lys Val Gly Glu Glu Thr Glu Thr Met Val Glu Lys
    1250                1255                1260
Leu Leu Thr Asn Trp Leu Ser Ile Cys Leu Tyr Ala Phe Leu Arg Glu
1265                1270                1275                1280
Val Ala Gly Glu Pro Leu Tyr Met Leu Phe Arg Ala Ile Gln Tyr Gln
                1285                1290                1295
Val Asp Lys Gly Pro Val Asp Ala Val Thr Gly Lys Ala Lys Arg Thr
            1300                1305                1310
Leu Asn Asp Ser Arg Leu Leu Arg Glu Asp Val Glu Phe Gln Pro Leu
        1315                1320                1325
Thr Leu Met Val Leu Val Gly Pro Gly Ala Gly Gly Ala Ala Gly Ser
    1330                1335                1340
Ser Glu Met Gln Arg Val Pro Ala Arg Val Leu Asp Thr Asp Thr Ile
1345                1350                1355                1360
Thr Gln Val Lys Glu Lys Val Leu Asp Gln Val Tyr Lys Gly Thr Pro
                1365                1370                1375
Phe Ser Gln Arg Pro Ser Val His Ala Leu Asp Leu Gly Glu Arg Ala
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Ser Pro Ala His Pro Pro Gln Gly Pro Phe Pro Thr Pro Pro Ala Pro
        1395                1400                1405
Gly Ala Pro Gln Leu Cys Leu Thr Met Asn Ile Pro Thr Leu Glu Asp
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Gly Glu Glu Gly Gly Val Cys Leu Trp His Leu Val Lys Ala Thr Glu
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Glu Pro Glu Gly Ala Lys Val Arg Cys Ser Ser Leu Arg Glu Arg Glu
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Pro Ala Arg Ala Lys Ala Ile Pro Glu Ile Tyr Leu Thr Arg Leu Leu
            1460                1465                1470
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Ser Met Lys Val Gly Ala Ala Trp Val Ala Gly Pro Glu Arg Arg Leu
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Ser Gln Gly Pro Arg Pro Ser Gln Gly Val Gly Gly Ala Gly Ala Ala
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Ser Ala Val Asp Gly Pro His Thr Leu Pro Ser Thr Gln Pro Leu Ser
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Pro Ala Ser Gln Gly Thr Leu Gln Lys Phe Val Asp Asp Thr Phe Gln
                1525                1530                1535
Ala Ile Leu Ser Val Asn Arg Pro Ile Pro Ile Ala Val Lys Tyr Leu
            1540                1545                1550
Phe Asp Leu Leu Asp Glu Leu Ala Glu Lys His Gly Ile Glu Asp Pro
        1555                1560                1565
Gly Thr Leu His Ile Trp Lys Thr Asn Ser Leu Leu Leu Arg Phe Trp
    1570                1575                1580
Val Asn Ala Leu Lys Asn Pro Gln Leu Ile Phe Asp Val Arg Val Ser
1585                1590                1595                1600
Asp Asn Val Asp Ala Ile Leu Ala Val Ile Ala Gln Thr Phe Ile Asp
                1605                1610                1615
Ser Cys Thr Thr Ser Glu His Lys Val Gly Arg Val Arg Ala Val Pro
            1620                1625                1630
Ala Ala Ala Ala Gly Arg Gly Leu Arg Arg Lys Gly Leu Trp Gly Lys
        1635                1640                1645
Pro Arg Gly Leu Cys Thr Glu Leu Trp Val Gly Ser Gly Ser Ile Met
    1650                1655                1660
Gly Ala Pro Ser Pro Pro Ser Ser Cys Leu Ala Pro Pro Leu Pro Pro
1665                1670                1675                1680
Glu Gln Asp Ser Pro Val Asn Lys Leu Leu Tyr Ala Arg Glu Ile Pro
                1685                1690                1695
Arg Tyr Lys Gln Met Val Glu Arg Tyr Tyr Ala Asp Ile Arg Gln Ser
            1700                1705                1710
Ser Pro Ala Ser Tyr Gln Glu Met Asn Ser Ala Leu Ala Glu Leu Ser
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Gly Asn Tyr Thr Ser Ala Pro His Cys Leu Glu Ala Leu Gln Glu Leu
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Tyr Asn His Ile His Arg Tyr Tyr Asp Gln Ile Ile Ser Ala Leu Glu
1745                1750                1755                1760
Glu Asp Pro Val Gly Gln Lys Leu Gln Leu Ala Cys Arg Leu Gln Gln
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            1780                1785
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<210> 5
<211> 5892
<212> DNA
<213> HOMO SAPIEN

<400> 5

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ctgctcctgg gggcggcgcg ggccggcgcc ctggagatcc agcgtcggtt cccctcgccc     180
acgcccacca acaacttcgc cctggacggc gcggcgggga ccgtgtacct ggcggccgtc     240
aaccgcctct atcagctgtc gggcgccaac ctgagcctgg aggccgaggc ggccgtgggc     300
ccggtgcccg acagcccgct gtgtcacgct ccgcagctgc cgcaggcctc gtgcgagcac     360
ccgcggcgcc tcacggacaa ctacaacaag atcctgcagc tggaccccgg ccagggcctg     420
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<210> 6
<211> 1925
<212> PRT
<213> HOMO SAPIEN

<400> 6

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            20                  25                  30
Leu Leu Leu Leu Leu Leu Leu Gly Ala Ala Arg Ala Gly Ala Leu Glu
        35                  40                  45
Ile Gln Arg Arg Phe Pro Ser Pro Thr Pro Thr Asn Asn Phe Ala Leu
    50                  55                  60
Asp Gly Ala Ala Gly Thr Val Tyr Leu Ala Ala Val Asn Arg Leu Tyr
65                  70                  75                  80
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Gln Leu Ser Gly Ala Asn Leu Ser Leu Glu Ala Glu Ala Ala Val Gly
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Pro Val Pro Asp Ser Pro Leu Cys His Ala Pro Gln Leu Pro Gln Ala
            100                 105                 110
Ser Cys Glu His Pro Arg Arg Leu Thr Asp Asn Tyr Asn Lys Ile Leu
        115                 120                 125
Gln Leu Asp Pro Gly Gln Gly Leu Val Val Val Cys Gly Ser Ile Tyr
    130                 135                 140
Gln Gly Phe Cys Gln Leu Arg Arg Arg Gly Asn Ile Ser Ala Val Ala
145                 150                 155                 160
Val Arg Phe Pro Pro Ala Ala Pro Pro Ala Glu Pro Val Thr Val Phe
                165                 170                 175
Pro Ser Met Leu Asn Val Ala Ala Asn His Pro Asn Ala Ser Thr Val
            180                 185                 190
Gly Leu Val Leu Pro Pro Ala Ala Gly Ala Gly Gly Ser Arg Leu Leu
        195                 200                 205
Val Gly Ala Thr Tyr Thr Gly Tyr Gly Ser Ser Phe Phe Pro Arg Asn
    210                 215                 220
Arg Ser Leu Glu Asp His Arg Phe Glu Asn Thr Pro Glu Ile Ala Ile
225                 230                 235                 240
Arg Ser Leu Asp Thr Arg Gly Asp Leu Ala Lys Leu Phe Thr Phe Asp
                245                 250                 255
Leu Asn Pro Ser Asp Asp Asn Ile Leu Lys Ile Lys Gln Gly Ala Lys
            260                 265                 270
Glu Gln His Lys Leu Gly Phe Val Ser Ala Phe Leu His Pro Ser Asp
        275                 280                 285
Pro Pro Pro Gly Ala Gln Ser Tyr Ala Tyr Leu Ala Leu Asn Ser Glu
    290                 295                 300
Ala Arg Ala Gly Asp Lys Glu Ser Gln Ala Arg Ser Leu Leu Ala Arg
305                 310                 315                 320
Ile Cys Leu Pro His Gly Ala Gly Gly Asp Ala Lys Lys Leu Thr Glu
                325                 330                 335
Ser Tyr Ile Gln Leu Gly Leu Gln Cys Ala Gly Gly Ala Gly Arg Gly
            340                 345                 350
Asp Leu Tyr Ser Arg Leu Val Ser Val Phe Pro Ala Arg Glu Arg Leu
        355                 360                 365
Phe Ala Val Phe Glu Arg Pro Gln Gly Ser Pro Ala Ala Arg Ala Ala
    370                 375                 380
Pro Ala Ala Leu Cys Ala Phe Arg Phe Ala Asp Val Arg Ala Ala Ile
385                 390                 395                 400
Arg Ala Ala Arg Thr Ala Cys Phe Val Glu Pro Ala Pro Asp Val Val
                405                 410                 415
Ala Val Leu Asp Ser Val Val Gln Gly Thr Gly Pro Ala Cys Glu Arg
            420                 425                 430
Lys Leu Asn Ile Gln Leu Gln Pro Glu Gln Leu Asp Cys Gly Ala Ala
        435                 440                 445
His Leu Gln His Pro Leu Ser Ile Leu Gln Pro Leu Lys Ala Thr Pro
    450                 455                 460
Val Phe Arg Ala Pro Gly Leu Thr Ser Val Ala Val Ala Ser Val Asn
465                 470                 475                 480
Asn Tyr Thr Ala Val Phe Leu Gly Thr Val Asn Gly Arg Leu Leu Lys
                485                 490                 495
Ile Asn Leu Asn Glu Ser Met Gln Val Val Ser Arg Arg Val Val Thr
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Val Ala Tyr Gly Glu Pro Val His His Val Met Gln Phe Asp Pro Ala
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Lys Val Ala Ala Cys Asn Val His Ser Thr Cys Gly Asp Cys Val Gly
545                 550                 555                 560
Ala Ala Asp Ala Tyr Cys Gly Trp Cys Ala Leu Glu Thr Arg Cys Thr
                565                 570                 575
Leu Gln Gln Asp Cys Thr Asn Ser Ser Gln Gln His Phe Trp Thr Ser
            580                 585                 590
Ala Ser Glu Gly Pro Ser Arg Cys Pro Ala Met Thr Val Leu Pro Ser
        595                 600                 605
Glu Ile Asp Val Arg Gln Glu Tyr Pro Gly Met Ile Leu Gln Ile Ser
    610                 615                 620
Gly Ser Leu Pro Ser Leu Ser Gly Met Glu Met Ala Cys Asp Tyr Gly
625                 630                 635                 640
Asn Asn Ile Arg Thr Val Ala Arg Val Pro Gly Pro Ala Phe Gly His
                645                 650                 655
Gln Ile Ala Tyr Cys Asn Leu Leu Pro Arg Asp Gln Phe Pro Pro Phe
            660                 665                 670
Pro Pro Asn Gln Asp His Val Thr Val Glu Met Ser Val Arg Val Asn
        675                 680                 685
Gly Arg Asn Ile Val Lys Ala Asn Phe Thr Ile Tyr Asp Cys Ser Arg
    690                 695                 700
Thr Ala Gln Val Tyr Pro His Thr Ala Cys Thr Ser Cys Leu Ser Ala
705                 710                 715                 720
Gln Trp Pro Cys Phe Trp Cys Ser Gln Gln His Ser Cys Val Ser Asn
                725                 730                 735
Gln Ser Arg Cys Glu Ala Ser Pro Asn Pro Thr Ser Pro Gln Asp Cys
            740                 745                 750
Pro Arg Thr Leu Leu Ser Pro Leu Ala Pro Val Pro Thr Gly Gly Ser
        755                 760                 765
Gln Asn Ile Leu Val Pro Leu Ala Asn Thr Ala Phe Phe Gln Gly Ala
    770                 775                 780
Ala Leu Glu Cys Ser Phe Gly Leu Glu Glu Ile Phe Glu Ala Val Trp
785                 790                 795                 800
Val Asn Glu Ser Val Val Arg Cys Asp Gln Val Val Leu His Thr Thr
                805                 810                 815
Arg Lys Ser Gln Val Phe Pro Leu Ser Leu Gln Leu Lys Gly Arg Pro
            820                 825                 830
Ala Arg Phe Leu Asp Ser Pro Glu Pro Met Thr Val Met Val Tyr Asn
        835                 840                 845
Cys Ala Met Gly Ser Pro Asp Cys Ser Gln Cys Leu Gly Arg Glu Asp
    850                 855                 860
Leu Gly His Leu Cys Val Trp Ser Asp Gly Cys Arg Leu Arg Gly Pro
865                 870                 875                 880
Leu Gln Pro Met Ala Gly Thr Cys Pro Ala Pro Glu Ile Arg Ala Ile
                885                 890                 895
Glu Pro Leu Ser Gly Pro Leu Asp Gly Gly Thr Leu Leu Thr Ile Arg
            900                 905                 910
Gly Arg Asn Leu Gly Arg Arg Leu Ser Asp Val Ala His Gly Val Trp
        915                 920                 925
Ile Gly Gly Val Ala Cys Glu Pro Leu Pro Asp Arg Tyr Thr Val Ser
    930                 935                 940
Glu Glu Ile Val Cys Val Thr Gly Pro Ala Pro Gly Pro Leu Ser Gly
945                 950                 955                 960
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Ser Tyr Val Leu Pro Leu Val His Ser Leu Glu Pro Thr Met Gly Pro
980 985 990
Lys Ala Gly Gly Thr Arg Ile Thr Ile His Gly Asn Asp Leu His Val
995 1000 1005
Gly Ser Glu Leu Gln Val Leu Val Asn Asp Thr Asp Pro Cys Thr Glu
1010 1015 1020
Leu Met Arg Thr Asp Thr Ser Ile Ala Cys Thr Met Pro Glu Gly Ala
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1075 1080 1085
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1155 1160 1165
Arg Leu Asp Tyr Leu Pro Asn Pro Gln Phe Ser Thr Ala Lys Arg Glu
1170 1175 1180
Lys Trp Ile Lys His His Pro Gly Glu Pro Leu Thr Leu Val Ile His
1185 1190 1195 1200
Lys Glu Gln Asp Ser Leu Gly Leu Gln Ser His Glu Tyr Arg Val Lys
1205 1210 1215
Ile Gly Gln Val Ser Cys Asp Ile Gln Ile Val Ser Asp Arg Ile Ile
1220 1225 1230
His Cys Ser Val Asn Glu Ser Leu Gly Ala Ala Val Gly Gln Leu Pro
1235 1240 1245
Ile Thr Ile Gln Val Gly Asn Phe Asn Gln Thr Ile Ala Thr Leu Gln
1250 1255 1260
Leu Gly Gly Ser Glu Thr Ala Ile Ile Val Ser Ile Val Ile Cys Ser
1265 1270 1275 1280
Val Leu Leu Leu Leu Ser Val Val Ala Leu Phe Val Phe Cys Thr Lys
1285 1290 1295
Ser Arg Arg Ala Glu Arg Tyr Trp Gln Lys Thr Leu Leu Gln Met Glu
1300 1305 1310
Glu Met Glu Ser Gln Ile Arg Glu Glu Ile Arg Lys Gly Phe Ala Glu
1315 1320 1325
Leu Gln Thr Asp Met Thr Asp Leu Thr Lys Glu Leu Asn Arg Ser Gln
1330 1335 1340
Gly Ile Pro Phe Leu Glu Tyr Lys His Phe Val Thr Arg Thr Phe Phe
1345 1350 1355 1360
Pro Lys Cys Ser Ser Leu Tyr Glu Glu Arg Tyr Val Leu Pro Ser Gln
1365 1370 1375
Thr Leu Asn Ser Gln Gly Ser Ser Gln Ala Gln Glu Thr His Pro Leu
1380 1385 1390
Leu Gly Glu Trp Lys Ile Pro Glu Ser Cys Arg Pro Asn Met Glu Glu

```
        1395                1400                1405
Gly Ile Ser Val Phe Ser Ser Leu Leu Asn Asn Lys His Phe Leu Ile
    1410                1415                1420
Val Phe Val His Ala Leu Glu Gln Gln Lys Asp Phe Ala Val Arg Asp
1425                1430                1435                1440
Arg Cys Ser Leu Ala Ser Leu Leu Thr Ile Ala Leu His Gly Lys Leu
                1445                1450                1455
Glu Tyr Tyr Thr Ser Ile Met Lys Glu Leu Leu Val Asp Leu Ile Asp
            1460                1465                1470
Ala Ser Ala Ala Lys Asn Pro Lys Leu Met Leu Arg Arg Thr Glu Ser
        1475                1480                1485
Val Val Glu Lys Met Leu Thr Asn Trp Met Ser Ile Cys Met Tyr Ser
    1490                1495                1500
Cys Leu Arg Glu Thr Val Gly Glu Pro Phe Phe Leu Leu Leu Cys Ala
1505                1510                1515                1520
Ile Lys Gln Gln Ile Asn Lys Gly Ser Ile Asp Ala Ile Thr Gly Lys
                1525                1530                1535
Ala Arg Tyr Thr Leu Asn Glu Glu Trp Leu Leu Arg Glu Asn Ile Glu
            1540                1545                1550
Ala Lys Pro Arg Asn Leu Asn Val Ser Phe Gln Gly Cys Gly Met Asp
        1555                1560                1565
Ser Leu Ser Val Arg Ala Met Asp Thr Asp Thr Leu Thr Gln Val Lys
    1570                1575                1580
Glu Lys Ile Leu Glu Ala Phe Cys Lys Asn Val Pro Tyr Ser Gln Trp
1585                1590                1595                1600
Pro Arg Ala Glu Asp Val Asp Leu Glu Trp Phe Ala Ser Ser Thr Gln
                1605                1610                1615
Ser Tyr Ile Leu Arg Asp Leu Asp Asp Thr Ser Val Val Glu Asp Gly
            1620                1625                1630
Arg Lys Lys Leu Asn Thr Leu Ala His Tyr Lys Ile Pro Glu Gly Ala
        1635                1640                1645
Ser Leu Ala Met Ser Leu Ile Asp Lys Lys Asp Asn Thr Leu Gly Arg
    1650                1655                1660
Val Lys Asp Leu Asp Thr Glu Lys Tyr Phe His Leu Val Leu Pro Thr
1665                1670                1675                1680
Asp Glu Leu Ala Glu Pro Lys Lys Ser His Arg Gln Ser His Arg Lys
                1685                1690                1695
Lys Val Leu Pro Glu Ile Tyr Leu Thr Arg Leu Leu Ser Thr Lys Gly
            1700                1705                1710
Thr Leu Gln Lys Phe Leu Asp Asp Leu Phe Lys Ala Ile Leu Ser Ile
        1715                1720                1725
Arg Glu Asp Lys Pro Pro Leu Ala Val Lys Tyr Phe Phe Asp Phe Leu
    1730                1735                1740
Glu Glu Gln Ala Glu Lys Arg Gly Ile Ser Asp Pro Asp Thr Leu His
1745                1750                1755                1760
Ile Trp Lys Thr Asn Ser Leu Pro Leu Arg Phe Trp Val Asn Ile Leu
                1765                1770                1775
Lys Asn Pro Gln Phe Val Phe Asp Ile Asp Lys Thr Asp His Ile Asp
            1780                1785                1790
Ala Cys Leu Ser Val Ile Ala Gln Ala Phe Ile Asp Ala Cys Ser Ile
        1795                1800                1805
Ser Asp Leu Gln Leu Gly Lys Asp Ser Pro Thr Asn Lys Leu Leu Tyr
    1810                1815                1820
Ala Lys Glu Ile Pro Glu Tyr Arg Lys Ile Val Gln Arg Tyr Tyr Lys
1825                1830                1835                1840
```

```
Gln Ile Gln Asp Met Thr Pro Leu Ser Glu Gln Glu Met Asn Ala His
                1845                1850                1855
Leu Ala Glu Glu Ser Arg Lys Tyr Gln Asn Glu Phe Asn Thr Asn Val
            1860                1865                1870
Ala Met Ala Glu Ile Phe Arg Ser Pro Lys Arg Tyr Arg Pro Gln Ile
        1875                1880                1885
Met Ala Ala Leu Glu Ala Asn Pro Thr Ala Arg Arg Thr Gln Leu Gln
    1890                1895                1900
His Lys Phe Glu Gln Val Val Ala Leu Met Glu Asp Asn Ile Tyr Glu
1905                1910                1915                1920
Cys Tyr Ser Glu Ala
                1925
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<210> 7
<211> 601
<212> DNA
<213> HOMO SAPIEN

<400> 7

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caccagagtc cctgtggagt cctgtggtca gtatcagagc tgcggcgagt gccttggctc      60
aggcgacccc cactgtggct ggtgtgtgct gcacaacact tgcacccgga aggagcggtg     120
tgagcggtcc aaggagcccc gcaggtttgc ctcggagatg aagcagtgtg tccggctgac     180
ggtccatccc aacaatatct ccgtctctca gtacaacgcg ctgctggtcc tggagacgta     240
caatgtcccg gagctgtcag ctggcgtcaa ctgcaccttt gaggacctgt cagagatgga     300
tgggctggtc gtgggcaatc agatccagtg ctactccct gcagccaagg aggtgccccg     360
gatcatcaca gagaatgggg accaccatgt cgtacagctt cagctcaaat caaaggagac     420
cggcatgacc ttcgccagca ccagctttgt cttctacaat tgcagcgtcc acaattcgtg     480
cctgtcctgc gtggagagtc ataccgctg ccactggtgt aaataccggc atgtctgcac     540
ccatgacccc aagacctgct ccttccagga aggccgagtg aagctgcccg aggtaggtcc     600
c                                                                     601
```

<210> 8
<211> 199
<212> PRT
<213> HOMO SAPIEN

<400> 8

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Thr Arg Val Pro Val Glu Ser Cys Gly Gln Tyr Gln Ser Cys Gly Glu
 1               5                  10                  15
Cys Leu Gly Ser Gly Asp Pro His Cys Gly Trp Cys Val Leu His Asn
            20                  25                  30
Thr Cys Thr Arg Lys Glu Arg Cys Glu Arg Ser Lys Glu Pro Arg Arg
        35                  40                  45
Phe Ala Ser Glu Met Lys Gln Cys Val Arg Leu Thr Val His Pro Asn
    50                  55                  60
Asn Ile Ser Val Ser Gln Tyr Asn Ala Leu Leu Val Leu Glu Thr Tyr
65                  70                  75                  80
Asn Val Pro Glu Leu Ser Ala Gly Val Asn Cys Thr Phe Glu Asp Leu
                85                  90                  95
Ser Glu Met Asp Gly Leu Val Val Gly Asn Gln Ile Gln Cys Tyr Ser
            100                 105                 110
Pro Ala Ala Lys Glu Val Pro Arg Ile Ile Thr Glu Asn Gly Asp His
        115                 120                 125
His Val Val Gln Leu Gln Leu Lys Ser Lys Glu Thr Gly Met Thr Phe
    130                 135                 140
```

```
Ala Ser Thr Ser Phe Val Phe Tyr Asn Cys Ser Val His Asn Ser Cys
145                 150                 155                 160
Leu Ser Cys Val Glu Ser Pro Tyr Arg Cys His Trp Cys Lys Tyr Arg
                165                 170                 175
His Val Cys Thr Asp Pro Lys Thr Cys Ser Phe Gln Glu Gly Arg Val
            180                 185                 190
Lys Leu Pro Glu Val Gly Pro
        195
```

<210> 9
<211> 6408
<212> DNA
<213> HOMO SAPIEN

<400> 9

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atgcctgctc tgggcccagc tcttctccag gctctctggg ccgggtgggt cctcaccctc      60
cagccccttc caccaactgc attcactccc aatggcacgt atctgcagca cctggcaagg     120
gaccccacct caggcaccct ctacctgggg gctaccaact tcctgttcca gctgagccct     180
gggctgcagc tggaggccac agtgtccacc ggccctgtgc tagacagcag ggactgcctg     240
ccacctgtga tgcctgatga gtgcccccag gcccagccta ccaacaaccc gaatcagctg     300
ctcctggtga gcccaggggc cctggtggta tgcgggagcg tgcaccaggg ggtctgtgaa     360
cagcggcgcc tggggcagct cgagcagctg ctgctgcggc cagagcggcc tggggacaca     420
caatatgtgg ctgccaatga tcctgcggtc agcacggtgg ggctggtagc ccagggcttg     480
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gaggagacag ccaagctggc agtgggccgc ctctccgagt acagccacca cttcgtgagt     660
gcctttgcac gtggggccag cgcctacttc ctgttcctgc ggcgggacct gcaggctcag     720
tctagagctt ttcgtgccta tgtatctcga gtgtgtctcc gggaccagca ctactactcc     780
tatgtggagt tgcctctggc ctgcgaaggt ggccgctacg ggctgatcca ggctgcagct     840
gtggccacgt ccagggaggt ggcgcatggg gaggtgctct ttgcagcttt ctcctcggct     900
gcaccccccа ctgtgggccg gcccccatcg gcggctgctg gggcatctgg agcctctgcc     960
ctctgtgcct tccccctgga tgaggtggac cggcttgcta atcgcacgcg agatgcctgc    1020
tacacccggg agggtcgtgc tgaggatggg accgaggtgg cctacatcga gtatgatgtc    1080
aattctgact gtgcacagct gccagtggac accctggatg cttatccctg tggctcagac    1140
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ccatactcca cacagagcat ccagcagggg tctgcagtga gcagagacct caccttgat     1380
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tggtgcgtgc tccttggcag gtgcagtcgc cgttctgagt gctcgagggg ccagggccca    1560
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cagatcatca ctgccctgga ggaggatggc acggcccaga agatgcagct gggctatcgg    6360
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<210> 10
<211> 2135
<212> PRT
<213> HOMO SAPIEN

<400> 10

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Met Pro Ala Leu Gly Pro Ala Leu Leu Gln Ala Leu Trp Ala Gly Trp
 1               5                  10                  15
Val Leu Thr Leu Gln Pro Leu Pro Pro Thr Ala Phe Thr Pro Asn Gly
            20                  25                  30
Thr Tyr Leu Gln His Leu Ala Arg Asp Pro Thr Ser Gly Thr Leu Tyr
        35                  40                  45
Leu Gly Ala Thr Asn Phe Leu Phe Gln Leu Ser Pro Gly Leu Gln Leu
    50                  55                  60
Glu Ala Thr Val Ser Thr Gly Pro Val Leu Asp Ser Arg Asp Cys Leu
65                  70                  75                  80
Pro Pro Val Met Pro Asp Glu Cys Pro Gln Ala Gln Pro Thr Asn Asn
                85                  90                  95
Pro Asn Gln Leu Leu Leu Val Ser Pro Gly Ala Leu Val Val Cys Gly
            100                 105                 110
Ser Val His Gln Gly Val Cys Glu Gln Arg Arg Leu Gly Gln Leu Glu
        115                 120                 125
Gln Leu Leu Leu Arg Pro Glu Arg Pro Gly Asp Thr Gln Tyr Val Ala
    130                 135                 140
Ala Asn Asp Pro Ala Val Ser Thr Val Gly Leu Val Ala Gln Gly Leu
145                 150                 155                 160
Ala Gly Glu Pro Leu Leu Phe Val Gly Arg Gly Tyr Thr Ser Arg Gly
                165                 170                 175
Val Gly Gly Gly Ile Pro Pro Ile Thr Thr Arg Ala Leu Trp Pro Pro
            180                 185                 190
Asp Pro Gln Ala Ala Phe Ser Tyr Glu Glu Thr Ala Lys Leu Ala Val
        195                 200                 205
Gly Arg Leu Ser Glu Tyr Ser His His Phe Val Ser Ala Phe Ala Arg
    210                 215                 220
Gly Ala Ser Ala Tyr Phe Leu Phe Leu Arg Arg Asp Leu Gln Ala Gln
225                 230                 235                 240
Ser Arg Ala Phe Arg Ala Tyr Val Ser Arg Val Cys Leu Arg Asp Gln
                245                 250                 255
His Tyr Tyr Ser Tyr Val Glu Leu Pro Leu Ala Cys Glu Gly Gly Arg
            260                 265                 270
```

```
Tyr Gly Leu Ile Gln Ala Ala Ala Val Ala Thr Ser Arg Glu Val Ala
        275                 280                 285
His Gly Glu Val Leu Phe Ala Ala Phe Ser Ser Ala Ala Pro Pro Thr
    290                 295                 300
Val Gly Arg Pro Pro Ser Ala Ala Ala Gly Ala Ser Gly Ala Ser Ala
305                 310                 315                 320
Leu Cys Ala Phe Pro Leu Asp Glu Val Asp Arg Leu Ala Asn Arg Thr
                325                 330                 335
Arg Asp Ala Cys Tyr Thr Arg Glu Gly Arg Ala Glu Asp Gly Thr Glu
            340                 345                 350
Val Ala Tyr Ile Glu Tyr Asp Val Asn Ser Asp Cys Ala Gln Leu Pro
        355                 360                 365
Val Asp Thr Leu Asp Ala Tyr Pro Cys Gly Ser Asp His Thr Pro Ser
    370                 375                 380
Pro Met Ala Ser Arg Val Pro Leu Glu Ala Thr Pro Ile Leu Glu Trp
385                 390                 395                 400
Pro Gly Ile Gln Leu Thr Ala Val Ala Val Thr Met Glu Asp Gly His
                405                 410                 415
Thr Ile Ala Phe Leu Gly Asp Ser Gln Gly Gln Leu His Arg Val Tyr
            420                 425                 430
Leu Gly Pro Gly Ser Asp Gly His Pro Tyr Ser Thr Gln Ser Ile Gln
        435                 440                 445
Gln Gly Ser Ala Val Ser Arg Asp Leu Thr Phe Asp Gly Thr Phe Glu
    450                 455                 460
His Leu Tyr Val Met Thr Gln Ser Thr Leu Leu Lys Val Pro Val Ala
465                 470                 475                 480
Ser Cys Ala Gln His Leu Asp Cys Ala Ser Cys Leu Ala His Arg Asp
                485                 490                 495
Pro Tyr Cys Gly Trp Cys Val Leu Leu Gly Arg Cys Ser Arg Arg Ser
            500                 505                 510
Glu Cys Ser Arg Gly Gln Gly Pro Glu Gln Trp Leu Trp Ser Phe Gln
        515                 520                 525
Pro Glu Leu Gly Cys Leu Gln Val Ala Ala Met Ser Pro Ala Asn Ile
    530                 535                 540
Ser Arg Glu Glu Thr Arg Glu Val Phe Leu Ser Val Pro Asp Leu Pro
545                 550                 555                 560
Pro Leu Trp Pro Gly Glu Ser Tyr Ser Cys His Phe Gly Glu His Gln
                565                 570                 575
Ser Pro Ala Leu Leu Thr Gly Ser Gly Val Met Cys Pro Ser Pro Asp
            580                 585                 590
Pro Ser Glu Ala Pro Val Leu Pro Arg Gly Ala Asp Tyr Val Ser Val
        595                 600                 605
Ser Val Glu Leu Arg Phe Gly Ala Val Val Ile Ala Lys Thr Ser Leu
    610                 615                 620
Ser Phe Tyr Asp Cys Val Ala Val Thr Glu Leu Arg Pro Ser Ala Gln
625                 630                 635                 640
Cys Gln Ala Cys Val Ser Ser Arg Trp Gly Cys Asn Trp Cys Val Trp
                645                 650                 655
Gln His Leu Cys Thr His Lys Ala Ser Cys Asp Ala Gly Pro Met Val
            660                 665                 670
Ala Ser His Gln Ser Pro Leu Val Ser Pro Asp Pro Pro Ala Arg Gly
        675                 680                 685
Gly Pro Ser Pro Ser Pro Pro Thr Ala Pro Lys Ala Leu Ala Thr Pro
    690                 695                 700
Ala Pro Asp Thr Leu Pro Val Glu Pro Gly Ala Pro Ser Thr Ala Thr
```

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705                 710                 715                 720
Ala Ser Asp Ile Ser Pro Gly Ala Ser Pro Ser Leu Leu Ser Pro Trp
                725                 730                 735
Gly Pro Trp Ala Gly Ser Gly Ser Ile Ser Ser Pro Gly Ser Thr Gly
            740                 745                 750
Ser Pro Leu His Glu Glu Pro Ser Pro Pro Ser Pro Gln Asn Gly Pro
        755                 760                 765
Gly Thr Ala Val Pro Ala Pro Thr Asp Phe Arg Pro Ser Ala Thr Pro
    770                 775                 780
Glu Asp Leu Leu Ala Ser Pro Leu Ser Pro Ser Glu Val Ala Ala Val
785                 790                 795                 800
Pro Pro Ala Asp Pro Gly Pro Glu Ala Leu His Pro Thr Val Pro Leu
                805                 810                 815
Asp Leu Pro Pro Ala Thr Val Pro Ala Thr Thr Phe Pro Gly Ala Met
            820                 825                 830
Gly Ser Val Lys Pro Ala Leu Asp Trp Leu Thr Arg Glu Gly Gly Glu
        835                 840                 845
Leu Pro Glu Ala Asp Glu Trp Thr Gly Gly Asp Ala Pro Ala Phe Ser
    850                 855                 860
Thr Ser Thr Leu Leu Ser Gly Asp Gly Asp Ser Ala Glu Leu Glu Gly
865                 870                 875                 880
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/14420 A3

(51) International Patent Classification[7]: C12N 15/12, 15/62, 15/63, C07K 14/705, 16/28, C12P 21/02, A61K 38/17, 39/395, G01N 33/53

(21) International Application Number: PCT/US00/23365

(22) International Filing Date: 25 August 2000 (25.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/150,576 25 August 1999 (25.08.1999) US

(71) Applicants *(for all designated States except US)*: **UNIVERSITY OF TORINO** [IT/IT]; Department of Biomedical Sciences and Human Oncology, IRCC, SP 142, I-10060 Candiolo (IT). **REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 12th floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ARTIGIANI, Stefania** [IT/IT]; Corso Brunelleschi, 121/B, I-10100 Torino (IT). **COMOGLIO, Paolo, M.** [IT/IT]; Strada Valsalice, 183/8, I-10100 Torino (IT). **GOODMAN, Corey, S.** [US/US]; Regents of the University of California, 12th floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US). **TESIER-LAVIGNE, Marc** [US/US]; Regents of the University of California, 12th floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US). **TAMAGNONE, Luca** [IT/IT]; Corso Einaudi, 43, I-10129 Torino (IT).

(74) Agent: **COX, Niki, D.**; Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*

(88) Date of publication of the international search report:
10 May 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/14420 A3

(54) Title: NOVEL MEMBERS OF THE PLEXIN FAMILY AND USES THEREOF

(57) Abstract: The invention provides methods and compositions related to novel plexins. The polypeptides may be produced recombinantly from transformed host cells and from the disclosed plexin encoding nucleic acids or purified from human cells. The invention provides isolated plexin hybridization probes and primers capable of specifically hybridizing with the disclosed plexin genes, plexin-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in biopharmaceutical industry. The invention also provides novel plexin neuropilin multimeric receptor complexes for semaphorins and methods of use thereof, including but not limited to, the treatment and diagnosis of neurological disease and neuroregeneration, immune modulation, and viral and oncological diseases.

# II ERNATIONAL SEARCH REPORT

Int. .ional Application No
PCT/US 00/23365

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/62 C12N15/63 C07K14/705 C07K16/28
C12P21/02 A61K38/17 A61K39/395 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE, SCISEARCH, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EMBL DATABASE EMHUM4:HSAB2313; ACCESSION-NO: AB002313, 1 July 1997 (1997-07-01), XP002157964 | 1-5 |
| Y | the whole document & DATABASE SWALL:O15031; ACCESSION-NO: O15031, 1 January 1998 (1998-01-01), the whole document & NAGASE, T. ET AL.: "Prediction of the coding sequences of unidentified human genes. VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro" DNA RESEARCH, vol. 4, 1997, pages 141-150, XP002102085 page 142 -page 150 'Results and Discussion' figure 3; tables 1,2 | 6-9 |

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 6 August 2001 | 31.08.01 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Donath, C |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EMBL DATABASE EM_HUM:AB014520;<br>ACCESSION-NO.:AB014520,<br>15 July 1998 (1998-07-15), XP002173834 | 1-5 |
| Y | the whole document<br>& ISHIKAWA, K.-I. ET AL.: "Prediction of the coding sequences of unidentified human genes. X. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro"<br>DNA RESEARCH,<br>vol. 5, 30 June 1998 (1998-06-30), pages 169-176, XP002121149<br>page 172 -page 176<br>* 'Results and Discussion' *<br>figures 1,2; tables 1-3 | 6-9 |
| X | EMBL DATABASE EMHUM4:HS5211110;<br>ACCESSION-NO: U52111,<br>9 May 1996 (1996-05-09), XP002173835 | 1-5 |
| Y | page 12 -page 13<br>* Gene="PLXB3" and product="plexin-related protein" * | 6-9 |
| X | EMBL DATABASE EMHUM6:HSOCTPROT;<br>ACCESSION-NO: X87831,<br>6 February 1996 (1996-02-06), XP002173836<br>the whole document | 1-3 |
| X | EMBL DATABASE EM_OV:XLPLEX;<br>ACCESSION-NO:D38175,<br>25 August 1995 (1995-08-25), XP002173837<br>the whole document<br>& OHTA, K. ET AL.: "Plexin: a novel neuronal cell surface molecule taht mediates cell adhesion via a homophilic binding mechanism in the presence of calcium ions"<br>NEURON,<br>vol. 14, 1995, pages 1189-1199, XP001013227 | 1-3 |
| X | WO 99 04263 A (THE JOHN HOPKINS UNIVERSITY SCHOOL OF MEDICINE)<br>28 January 1999 (1999-01-28) | 10,11 |
| Y | page 5, line 9 -page 10, line 16<br>page 16, line 6 -page 22, line 3<br>page 23, line 22 -page 24, line 18<br>page 29, line 6 -page 32, line 4 | 12,13 |

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4

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| Y | KAMEYAMA, T. ET AL.: "Identification of a cell surface protein plexin (the B2) in mouse, and its expression in developing nervous systems" NEUROSCIENCE RESEARCH SUPPLEMENT, vol. 18, 1993, page S115 XP000945106 the whole document | 6-9 |
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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/23365

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 10,11 (partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 10-13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
Although claim 14 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.: 10,11 (partially)

Claims 10 and 11 concern a methods which comprise the administration of an agent capable of interfering with the association between a plexin and a neuropilin. Since in the specification this agent is exemplified only to be an antibody raised against the plexin and since it is completely unclear which kind of substances besides said antibody also will be capable of interfering with the association between a plexin and a neuropilin, the scope of said claims is totally ambiguous and undefined as far as any kind of substance other than an antibody raised against the plexin is concerned.
Therefore, the search in respect of claims 10 nad 11 has been limited to methods comprising the administration of an antibody raised against the plexin and which is capable of interfering with the assiciation between a plexin and a neuropilin.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-9,12-14 (partially)

Claims 1-9,12-14 (partially) refer to the isolation and cloning of a member of the plexin family, plexin B-2. Antibodies specifically binding to this polypeptide, a fusion protein, and methods of diagnosing for tumors, treating a disorder involving aberrant immune regulation involving a signal pathway between plexin and a neuropilin, and suppressing aberrant cell growth, all methods by using either the polypeptide or the antibodies directed against the plexin B-2.

2. Claims: 1-9,12-14 (partially)

Claims 1-9,12-14 (partially) refer to the isolation and cloning of a member of the plexin family, plexin B-3. Antibodies specifically binding to this polypeptide, a fusion protein, and methods of diagnosing for tumors, treating a disorder involving aberrant immune regulation involving a signal pathway between plexin and a neuropilin, and suppressing aberrant cell growth, all methods by using either the polypeptide or the antibodies directed against the plexin B-3.

3. Claims: 1-9,12-14 (partially)

Claims 1-9,12-14 (partially) refer to the isolation and cloning of a member of the plexin family, plexin D-1. Antibodies specifically binding to this polypeptide, a fusion protein, and methods of diagnosing for tumors, treating a disorder involving aberrant immune regulation involving a signal pathway between plexin and a neuropilin, and suppressing aberrant cell growth, all methods by using either the polypeptide or the antibodies directed against the plexin D-1.

4. Claims: 1-9,12-14 (partially)

Claims 1-9,12-14 (partially) refer to the isolation and cloning of a member of the plexin family, plexin A-4. Antibodies specifically binding to this polypeptide, a fusion protein, and methods of diagnosing for tumors, treating a disorder involving aberrant immune regulation involving a signal pathway between plexin and a neuropilin, and suppressing aberrant cell growth, all methods by using either the polypeptide or the antibodies directed against the plexin A-4.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. Claims: 10,11

Claims 10 and 11 refer either to a method of suppressing or altering aberrant cell growth involving a signalling pathway between a plexin and a neuropilin in a mammal or to a method of treating, suppressing or altering a disorder involving aberrant immune regulation involving a signalling pathway between a plexin and a neuropilin in a mammal, both methods comprises the administration of an agent in general being capable of interfering with the association between the plexin and neuropilin to said mammal.

# II ERNATIONAL SEARCH REPORT

Information on patent family members

Int. donal Application No
PCT/US 00/23365

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| WO 9904263 A | 28-01-1999 | AU 8405398 A | 10-02-1999 |